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Transient Elevation of Granulocyte Colony-Stimulating Factor Levels in Cerebrospinal Fluid at the Initial Stage of Aseptic Meningitis in Children

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ABSTRACT

At the early stage of aseptic meningitis, there is a transient increase in neutrophil counts in the cerebrospinal fluid. Some factors in the cerebrospinal fluid might induce migration of neutrophils into the cerebrospinal fluid. Granulocyte colony-stimulating factor (G-CSF) plays an important role, not only as a hemopoietic factor but also as a regulating factor for a biologic defense system by neutrophils in the foci of infection. To analyze the role of G-CSF on accumulating neutrophils in the cerebrospinal fluid, we have measured G-CSF levels in the cerebrospinal fluid of children with aseptic meningitis, paying particular attention to the phasal transition. Within the first 2 d from the onset, G-CSF levels in the cerebrospinal fluid were 223 ± 97 ng/L, significantly higher than those of the patients without meningitis ($p < 0.01$). Beyond the second day after the onset, the G-CSF levels rapidly decreased to below the detectable level, even though the patients manifested meningeal signs and symptoms. There was a direct relationship between G-CSF levels and neutrophil counts in the cerebrospinal fluid ($r = 0.763$, $p < 0.01$). During the first 2 d after the onset, the G-CSF level in the cerebrospinal fluid in each case was remarkably higher than that in the serum. This finding suggests that the G-CSF in the cerebrospinal fluid was produced in the spinal cavity. From our results, the transient elevation of G-CSF levels might lead to the transient increase in neutrophil counts in the cerebrospinal fluid by recruiting them from the peripheral blood at the initial stage of aseptic meningitis. (***Pediatr Res* 37: 160–164, 1995**)

Abbreviations

CSF, colony-stimulating factor
G-CSF, granulocyte colony-stimulating factor
rhG-CSF, recombinant human G-CSF
GM-CSF, granulocyte-macrophage colony-stimulating factor
IFN, interferon

Aseptic meningitis is characterized by an increase in mononuclear cells in the cerebrospinal fluid. However, at the early stage of the disease, the cerebrospinal fluid temporarily contains chiefly neutrophils (1). The pathogenesis of the increase in neutrophils in the cerebrospinal fluid remains unclear. Some factors in the cerebrospinal fluid might induce migration of neutrophils into the cerebrospinal fluid at the early stage of the disease.

G-CSF was found as a factor that stimulates the proliferation of precursor cells, specifically for granulocytic lineage (2–4). This cytokine acts not only on progenitor cells of granulocytic lineage but also directly on mature neutrophils (5–8). G-CSF activates the functions of mature neutrophils, including C3bi receptor expression (9), adherence (9, 10), superoxide release (11, 12), phagocytosis (13), antibody-dependent cell-mediated cytotoxicity (14, 15), and chemotactic activity (16); it also prolongs survival time (17, 18). Serum G-CSF levels have been reported to elevate in many inflammatory diseases, with an increase of neutrophils in the peripheral blood (19, 20). Therefore, G-CSF plays an important role, not only as a hemopoietic factor but also as a regulating factor for a biologic defense system in the foci of both infection and inflammation (21, 22). G-CSF might be involved in the development of inflammation in the focus by the activation of mature neutrophils. However, little is known about G-CSF levels in inflammatory foci such as synovia, pleural fluid, and cerebrospinal fluid.

To analyze the role of G-CSF in accumulating neutrophils in the cerebrospinal fluid during the early stage of aseptic meningitis, we have measured G-CSF levels in the cerebrospinal fluid of children with the disease. This work has demonstrated that G-CSF levels in the cerebrospinal fluid are markedly elevated in the acute phase of the illness.

METHODS

Study population. Fourteen patients with aseptic meningitis, ranging in age from 4 mo to 15 y, were admitted to our hospital from June to October 1991. The following criteria were used for the diagnosis of aseptic meningitis: *1*) all of the following

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symptoms: fever, headache, vomiting, and neck stiffness; *2*) cell counts greater than 35 per μL in the cerebrospinal fluid; and *3*) sterile cerebrospinal fluid found in bacteriologic studies. The first day of the illness was determined as the day when any of the symptoms of meningitis had first occurred. The following criteria were used for the diagnosis of no meningitis: *1*) cell counts less than 5 per μL in the cerebrospinal fluid, and *2*) sterile cerebrospinal fluid found in bacteriologic studies. Patients meeting these criteria were used as controls. The patients without meningitis included three with meningism due to upper respiratory tract infections, two with epilepsy, two with acute gastroenteritis, and one with febrile convulsions. They all received lumbar punctures to rule out meningitis. Table 1 shows the characteristics of 14 patients with aseptic meningitis and eight who did not have meningitis. The age, sex, and concentration of glucose in the cerebrospinal fluid in both groups were statistically similar. The protein values in the cerebrospinal fluid of the patients with meningitis were not significantly different from those of the group without meningitis. Some of the meningitis patients had elevations of protein values, but none of the patients without meningitis did.

Viral study. In the summer of 1991, a prevalence of echovirus 30 caused endemic aseptic meningitis. Serum antibodies for anti-echovirus 30 were examined by a serum neutralization test in pairs of samples collected 2 to 3 wk apart. In six of nine patients studied, the antibody titer rose four times or more. Furthermore, in three patients with meningitis and four patients without meningitis, viruses isolated from the cerebrospinal fluid were studied by using tissue culture microplates containing cynomolgus monkey kidney, Vero, and HeLa cells (22a). The isolated virus was determined by the neutralizing test with standard antivirus serum. In all three patients with meningitis studied, echovirus 30 was isolated, but in all four patients without meningitis studied, no virus was isolated. Therefore, it seems that the illness was caused by echovirus 30 in most of the patients with meningitis.

Sample collection. Samples of the cerebrospinal fluid were obtained by lumbar puncture after receiving informed consent for this study from the parents of the patients. The cerebrospinal fluid was immediately centrifuged at 150 $\times$ *g* for 10 min, and the supernatant was stored at -30°C. Blood samples were collected at the same time. Sera were isolated by centrifugation at 400 $\times$ *g* for 10 min and stored at -30°C.

Assay for G-CSF. Concentrations of G-CSF were measured by a subsequent ELISA. The assay was based on the dual antibody immunometric sandwich method, as previously described (23). Rabbit antiserum against rhG-CSF (Chugai Pharmaceuticals, Tokyo, Japan) (24) was precipitated with ammonium sulfate. The precipitate was dissolved in 0.05 M borate-buffered saline containing 0.9% NaCl (BBS) (pH 8.0) and fractionated to IgG by Ultrogel AcA44 gel filtration (LKB, Stockholm, Sweden). Polystyrene tubes (Nunc, Roskilde, Denmark) were coated with BBS containing 20 mg/L IgG for 16 h at 4°C. The coating solution was removed by aspiration, and the tubes were washed twice with BBS. Each 0.2-mL aliquot and standard rhG-CSF was dispensed into the tubes diluted with 0.5 mL of 0.05 M Tris-HCl buffer (pH 8.0) containing 0.25% BSA, 0.05% Tween 20, 2% polyethylene glycol, 0.9% NaCl, and 0.1% NaN_3. The tubes were incubated for 2 h at room temperature and washed three times with 20 mM Tris-HCl buffer. The tubes were filled with horseradish peroxidase–labeled anti–rhG-CSF rabbit Fab′ (Chugai Pharmaceuticals, Tokyo, Japan), incubated for 2 h at room temperature, and washed three times. The tubes were filled with 1 mL of substrate solution consisting of 0.1 M phosphate citrate buffer (pH 6.0), 0.015% hydrogen peroxide, and 3 g/L o-phenylene diamine (Nakarai Chemicals, Kyoto, Japan). After 1 h of incubation at room temperature in the dark, the enzyme reaction was stopped with 1 mL of 4N H_2SO_4, and each sample was measured for adsorbance at 492 nm in duplicate. The limit of detection was 30 ng/L. This assay system did not react with macrophage-CSF, GM-CSF, or IL-3.

Statistical analysis. Statistical significance of the data was analyzed with the Mann-Whitney U test. The relationships between cerebrospinal G-CSF levels and other indices of meningeal inflammation were estimated by Pearson's correlation coefficients. Differences were considered significant when the p value was less than 0.05.

RESULTS

G-CSF levels in cerebrospinal fluid. Concentrations of G-CSF were assayed within the first 2 d from the onset of aseptic meningitis in nine patients (Fig. 1), and the values were 223 $\pm$ 97 ng/L (mean $\pm$ SD, range 53 to 386 ng/L). These levels were significantly higher ($p < 0.01$) than those of the children with no meningitis (all were less than 30 ng/L). The anti-echovirus 30 antibody titer rose 4 times or more in six patients but not in three patients. The G-CSF levels in the former were 217 $\pm$ 59 ng/L and those in the latter were 235 $\pm$ 169 ng/L. These values were not statistically significant. Figure 2 illustrates time-dependent changes in G-CSF levels in the cerebrospinal fluid. After the second day of the onset of the disease, the G-CSF values rapidly decreased to below the detectable level, even though the patients manifested meningeal symptoms and signs: fever, headache, vomiting, and neck stiffness. The G-CSF levels sequentially dropped in all patients examined longitudinally, as shown in Figure 2. G-CSF levels

Table 1. *Characteristics of study population*

	Age (y)		Sex	Total protein in CSF* (g/L)	Glucose in CSF* (mmol/L)
	Mean $\pm$ SD	Range	(M/F)	Mean $\pm$ SD	Mean $\pm$ SD
Meningitis	7.2 $\pm$ 4.6	0.3–15.8	10/4	0.205 $\pm$ 0.104	3.52 $\pm$ 0.65
No meningitis	8.3 $\pm$ 6.3	0.1–13.5	4/4	0.206 $\pm$ 0.088	3.66 $\pm$ 1.00

* CSF, cerebrospinal fluid.

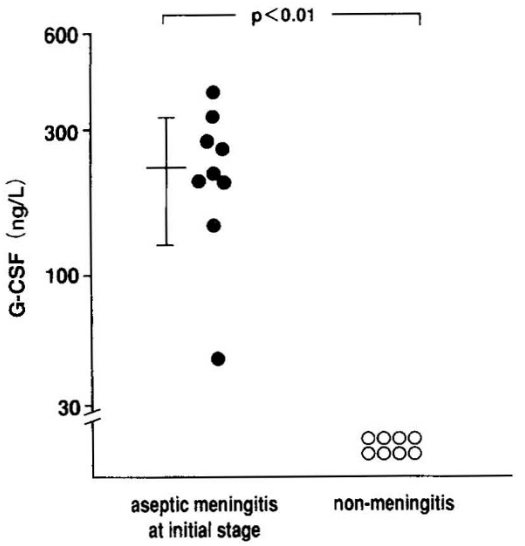

Figure 1. Levels of G-CSF in the cerebrospinal fluid of the patients within the first 2 d of onset of aseptic meningitis (●) and the patients without meningitis (○). The horizontal bar shows mean and the vertical bar shows SD in the meningitis group.

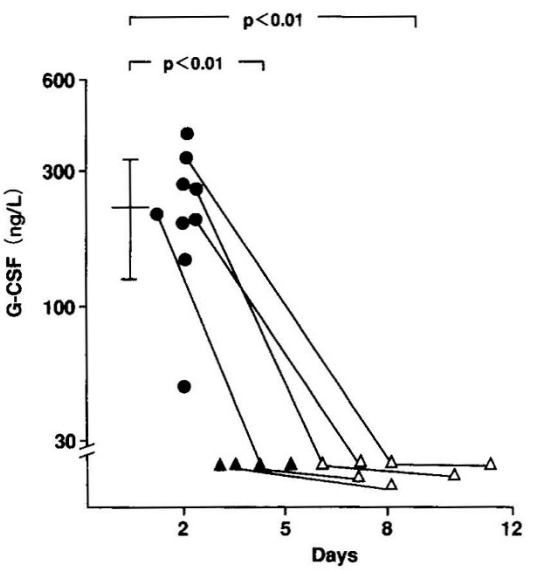

Figure 2. Levels of G-CSF in the cerebrospinal fluid from the onset of meningeal symptoms. *Black symbols* represent samples from the patients with meningeal signs or symptoms, and *open symbols* represent those without meningeal signs and symptoms. *Circles* represent samples within the first 2 d of aseptic meningitis, and *triangles* represent those after the second day. The horizontal bar shows mean and the vertical bar shows SD of *black circles*. Tie bars connect the samples of the same patients.

within the first 2 d of the illness were significantly higher than those after the second day ($p < 0.01$).

G-CSF levels in sera. G-CSF was not detectable in any serum samples except for one patient's sample, which had 33 ng/L on the second day of the illness. The G-CSF levels of sera were statistically lower ($p < 0.01$) than those of the cerebrospinal fluid on any day of the disease in each patient (Table 2).

Neutrophil counts in the cerebrospinal fluid. Within the first 2 d of the illness, neutrophil counts increased (range 6 to 148 per μL) in the cerebrospinal fluid of all patients (Fig. 3) and particularly accounted for the majority of cells in six of

Table 2. *G-CSF levels in cerebrospinal fluid and serum in aseptic meningitis*

	Within first 2 d	After second day with signs or symptoms	After second day without signs or symptoms
CSF*	223 ± 9.7† (n = 9)	<30 (n = 4)	<30 (n = 7)
Serum	33 (n = 1) <30 (n = 5)	<30 (n = 3)	<30 (n = 3)

Values are expressed in ng/L of G-CSF.

* CSF, cerebrospinal fluid.

† Value is represented as mean ± SD. G-CSF levels are statistically higher in the cerebrospinal fluid than in the serum ($p < 0.01$).

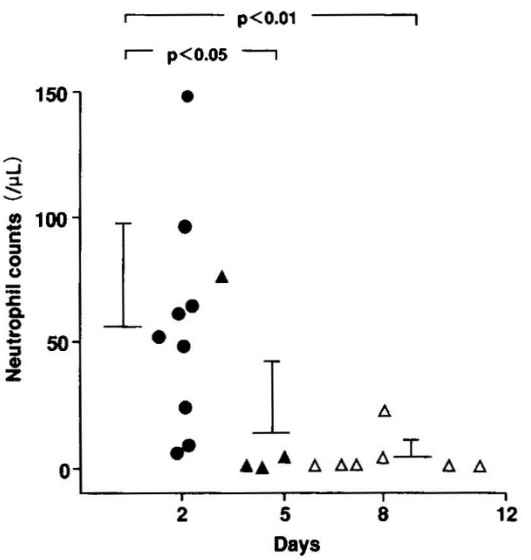

Figure 3. Neutrophil counts in the cerebrospinal fluid of the patients with aseptic meningitis. *Black symbols* represent samples from the patients with meningeal signs or symptoms, and *open symbols* represent those without meningeal signs and symptoms. *Circles* represent samples taken within the first 2 d of the illness, and *triangles* represent those taken after the second day. Horizontal bars show means and vertical bars show SD in each group.

nine patients. After the fourth day of the disease, neutrophils disappeared rapidly and were replaced by mononuclear cells.

Relationship between G-CSF levels and clinical indices. Figure 4 shows the relationship between the G-CSF levels and neutrophil counts in the cerebrospinal fluid of the patients. At the initial stage of the disease, both G-CSF levels and neutrophil counts increased. Thereafter, neutrophils disappeared from the cerebrospinal fluid as G-CSF levels decreased. There was a direct relationship between G-CSF levels and neutrophil counts ($r = 0.763$, $p < 0.01$). However, no distinct correlation was found between G-CSF levels and mononuclear cell counts in the cerebrospinal fluid ($r = -0.446$, $p > 0.05$). The G-CSF concentrations in the cerebrospinal fluid did not correlate with either the level or duration of fever, headache, or vomiting; glucose levels; or protein values in the cerebrospinal fluid.

DISCUSSION

We have investigated whether G-CSF levels correlate with neutrophil counts in the cerebrospinal fluid in aseptic meningitis, paying particular attention to their changes in the course of the disease. The G-CSF levels in the cerebrospinal fluid

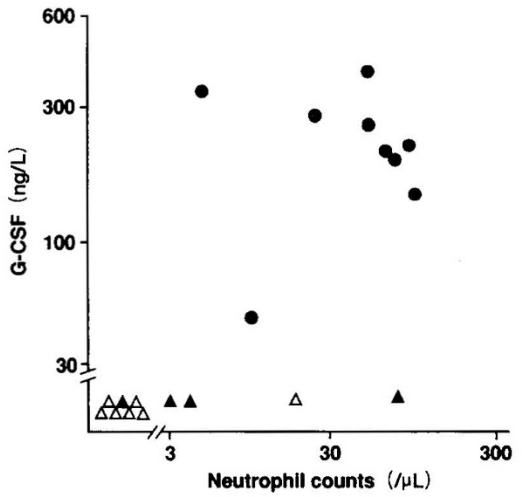

Figure 4. Relationship between the level of G-CSF and neutrophil counts in the cerebrospinal fluid of the patients. For all values, the Pearson's correlation coefficient is 0.763 ($p < 0.01$). *Black symbols* represent samples from the patients with meningeal signs or symptoms, and *open symbols* represent those without meningeal signs and symptoms. *Circles* represent samples taken within the first 2 d of the illness, and *triangles* represent those taken after the second day.

significantly increased at the initial stage of aseptic meningitis. However, on and after the third day of the illness, the G-CSF levels decreased rapidly to points below sensitivity (<30 ng/L), regardless of the presence of any meningeal sign or symptom. It is now clear that in cases of aseptic meningitis G-CSF does not vanish from the cerebrospinal fluid with the disappearance of meningeal signs and symptoms; rather, it appears only during the initial stage of the illness.

Shimoda *et al.* (25) have reported a rise in the level of G-CSF in the cerebrospinal fluid in 12 (67%) of 18 cases of aseptic meningitis. In contrast, we have found elevation of the G-CSF levels in every case during the first 2 d. Although sensitivity of measurement was almost the same in both of the assays (their sensitivity level was 20 ng/L), the frequency of G-CSF elevation in their subjects seems lower than in ours. We did not detect G-CSF in any patient whom we first punctured after the second day from onset. They did not present the data of G-CSF kinetics in the cerebrospinal fluid in aseptic meningitis. Inasmuch as the G-CSF levels in aseptic meningitis decreased rapidly during the first 3 d after onset, they may not have found G-CSF elevation in any of their subjects.

We next studied how G-CSF increased in the cerebrospinal fluid of patients with aseptic meningitis. At the initial stage, G-CSF levels in the cerebrospinal fluid were remarkably higher than those in the serum at the same time in each case. Serum G-CSF levels did not fluctuate during the progression of the illness. These results suggest that elevated levels of G-CSF in the cerebrospinal fluid were not caused by a switch over from the blood but rather by its production in the focus of inflammation, *i.e.* in the spinal cavity. Monocytes/macrophages (26–28), endothelial cells (29, 30), and fibroblasts (31, 32) produce G-CSF. Astrocytes (33–35) also produce G-CSF in the CNS. It is reported that IL-1β (36), IL-6 (37, 38), IFN-γ (38), and GM-CSF (39), but not tumor necrosis factor-α (36, 40), in the cerebrospinal fluid are increased in aseptic meningitis. IL-1β can stimulate fibroblasts (32), endothelial cells (29, 30), and astrocytes (33, 35) and GM-CSF can stimulate monocytes/macrophages (28) to produce G-CSF *in vitro*. It is unlikely that endotoxin stimulates production of G-CSF in aseptic meningitis. Taken together, we assume there are cytokine networks in the CNS: IL-1β and GM-CSF induced by viral infection might stimulate fibroblasts, endothelial cells, monocytes/macrophages, and astrocytes to produce G-CSF.

We analyzed the relationship in the cerebrospinal fluid between the G-CSF levels and neutrophil counts. As is well known, neutrophils increase transiently during the initial stage of the illness. In our studies, both the G-CSF levels and neutrophil counts rose in the cerebrospinal fluid at the initial stage, and afterward the neutrophil counts decreased along with the drop in the G-CSF levels. G-CSF can augment migration of mature neutrophils (16). We conjecture that the G-CSF increasing in the cerebrospinal fluid can induce mobilization of neutrophils out of the peripheral blood and into the spinal cavity. It deserves discussion why the appearance of neutrophils in the cerebrospinal fluid takes place only during the initial stage of the illness. The plasma terminal half-life of rhG-CSF is 7.2 $\pm$ 3.7 h when G-CSF is injected i.v. at 100, 200, 400, and 800 $\mu g/m^2$ (41). This short half-life of G-CSF causes rapid disappearance once G-CSF production stops in the focus of inflammation. Furthermore, the period of viral proliferation in the meninges seems to be short, and there is no endotoxin present as a strong stimulator of G-CSF production in the cerebrospinal fluid in viral infection. In any event, it is likely that such a rapid change in the G-CSF levels results in transient increases in the neutrophil counts in the cerebrospinal fluid during only the initial stage of aseptic meningitis.

Activated mature neutrophils produce chemical agents, including leukotrienes, IFN, etc. (42). Inasmuch as leukotriene B_4 increases vascular permeability and develops inflammatory reactions, it can lead to the subsequent migration of lymphocytes into the spinal cavity. IFN also may act as a defense mechanism against viruses. However, additional studies are necessary to elucidate the exact pathophysiologic significance of the transient increase in neutrophils in the cerebrospinal fluid along with elevation of G-CSF levels.

Pleocytosis is an index for meningitis. However, it is difficult at the initial stage to distinguish aseptic meningitis from purulent meningitis by neutrophil counts alone. G-CSF in the cerebrospinal fluid increases in aseptic meningitis. The peak levels reach 386 ng/L as shown in our study or 366 ng/L as reported by others (25). We also measured G-CSF levels in the cerebrospinal fluid in a case of pneumococcal purulent meningitis. The G-CSF levels were 4700 ng/L on the third day of illness, 111 ng/L on the fifth day, and below sensitivity on the seventh day, when meningeal symptoms had disappeared. Shimoda *et al.* (25) reported that G-CSF levels in the cerebrospinal fluid markedly increased and averaged 1500 ng/L in 10 of 11 cases of purulent meningitis. These levels—in both their investigation (25) and ours—in patients with purulent meningitis were significantly higher ($p < 0.05$) than those in our patients with aseptic meningitis. In five of their subjects (25), the G-CSF values dropped below sensitivity after the disappearance of meningeal symptoms. The highly elevated levels

of G-CSF persist for a long time, probably because an endotoxin derived from bacteria exists as a powerful stimulating factor of G-CSF production. The extremely high levels of G-CSF in the cerebrospinal fluid over 560 ng/L (mean + 3.5 SD in our patients with aseptic meningitis) may be suggestive of purulent meningitis. Furthermore, disappearance of G-CSF from the cerebrospinal fluid within a few days may allow us to suppose aseptic meningitis. Taken together, G-CSF levels in the cerebrospinal fluid may be a useful index of meningitis. We look forward to the development of a rapid and simple method to assay G-CSF levels.

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