Levels of Serum Granulocyte Colony-Stimulating Factor in Patients With Infections

By Mutsumi Kawakami, Hisashi Tsutsumi, Toshiro Kumakawa, Hiroki Abe, Makiko Hirai, Shinichiro Kurosawa, Mayumi Mori, and Masafumi Fukushima

To clarify the physiologic roles of granulocyte colony-stimulating factor (G-CSF) in infectious states in vivo, we examined the serum levels of G-CSF in patients with infection. Serum samples from 24 patients in the acute stage of infection (14 men and 10 women, age 65 to 101, without hematologic disorders), as well as samples from 32 age-matched normal elderly volunteers were investigated. Sixteen of the initial 24 patients were reexamined after the recovery phase. G-CSF levels were examined by quantitative enzyme immunoassay. The G-CSF level in normal elderly controls, 25.3 ± 19.7 pg/mL, was not different from that reported in other findings. There was no statistically significant relationship between their G-CSF level and peripheral white blood cell count or neutrophilic granulocyte count. The G-CSF level in the acute stage of infection was 731.8 ± 895.0 pg/mL, with a range of 30 to 3,199 pg/mL. There was no significant difference in G-CSF levels between patients with respiratory tract infection and those with urinary tract infection. In all 16 cases examined, the serum G-CSF level in the acute stage of infection was significantly higher than that after recovery phase, the latter being the same as the level in normal elderly controls. G-CSF must therefore play a significant role in human infectious states in vivo.

G-Granulocyte colony-stimulating factor (G-CSF) has been identified as a glycoprotein that stimulates the production and functional activation of neutrophilic granulocytes both in vivo and in vitro.1-4 In 1989 Watari et al.7 reported serum G-CSF levels in 56 normal healthy volunteers and in various hematologic disorders. However, the kinetics and pathophysiologic roles of G-CSF have not been elucidated. In infectious states, increased levels of G-CSF particularly in cases of bacterial infection, but in only two acquired immunodeficiency syndrome (AIDS) cases. There has been no report on the G-CSF serum levels in infections without hematologic disorders, other malignancies or immunologic disorders. In this study we investigated the G-CSF levels in infectious states without hematologic disorders, other malignancies or immunologic disorders.

MATERIALS AND METHODS

Sera. Serum samples, obtained as soon as possible after the onset of infection and before the administration of antibiotics and/or anti-inflammatory agents, from patients in the acute stage of infection were examined. The patients consisted of 24 Japanese individuals age 65 to 101, 14 men and 10 women. Some of them had complicated old cerebral vascular disease, hypertension, and/or diabetes mellitus but no hematologic or immunologic disorders or other malignancies. Serum samples after recovery phase were also investigated in 16 of these 24 patients.

Serum was examined from 32 elderly normal healthy volunteers as age-matched controls (age 64 to 88, 8 men and 24 women). The daily profile of serum G-CSF levels was examined in 3 persons. Serum samples were obtained at 6 AM, 9:30 AM, 11:30 AM, 2:30 PM, 4:30 PM and 8 PM. The serum was separated by centrifugation after collection, and stored frozen at −80°C until use.

Serum G-CSF levels were determined by quantitative enzyme immunoassay (EIA) as described by Motojima et al.6 Briefly, 200 μL of each sample and its three dilutions (3:4, 1:2, 1:4), or serial dilutions of recombinant G-CSF, together with 500 μL of EIA buffer containing 2% polyethylene glycol (molecular weight: 6,000), were added to a polystyrene tube coated with rabbit anti-G-CSF IgG. After 2 hours of incubation at room temperature, 100 μL of HRP-conjugated anti-G-CSF Fab' solution was added and further incubated for 2 hours at room temperature. After washing three times with 20 mmol/L Tris-HCl, pH 8.0, containing 0.005% benzalkonium chloride, 1 mL of reaction mixture (3 mg/mL o-phenylenediamine dihydrochloride, 2.3% (wt/vol) disodium hydrogenphosphate, 0.38% citric acid, 0.1% salicylic acid, and 0.015% H2O2) was added for the color reaction. After incubation for 1 hour at room temperature in the dark, the reaction was stopped by adding 1 mL of 4 N sulfuric acid, and the resulting optical density was measured at 492 nm using a Hitachi spectrophotometer (Hitachi, Tokyo, Japan).

RESULTS

G-CSF levels in elderly controls. The G-CSF level in the 32 normal healthy controls was 25.3 ± 19.7 pg/mL, and there was no difference due to sex or age (Table 1). The G-CSF level was less than 40 pg/mL in 28 (87.5%) of the controls, and less than 100 pg/mL in all cases. There was no statistically significant relationship between G-CSF level and peripheral white blood cell (WBC) count or neutrophilic granulocyte count as shown in Figs 1A and B. There were no significant changes in the concentration of serum G-CSF during one day (Fig 2).

G-CSF levels in patients with infection. The G-CSF level in the acute phase of infection was 731.8 ± 895.0 pg/mL, with a range of 30 to 3,199 pg/mL. The WBC count

Table 1. Serum G-CSF Levels in Normal Elderly Volunteers

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No.</th>
<th>G-CSF pg/mL</th>
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<tbody>
<tr>
<td>Total</td>
<td>32</td>
<td>25.3 ± 19.7</td>
</tr>
<tr>
<td>Male</td>
<td>8</td>
<td>25.1 ± 18.3</td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>25.3 ± 20.6</td>
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<tr>
<td>Over Age 75</td>
<td>14</td>
<td>28.8 ± 22.3</td>
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<tr>
<td>Under Age 75</td>
<td>18</td>
<td>22.6 ± 17.7</td>
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in the acute phase was \(13,842.9 \pm 4,644.3\) \(\text{pg/mL}\). All patients needed antibiotic agents, and it took from 1 to 8 days to lower the fever. The highest level of c-reactive protein during infection was \(10.6 \text{ to } 32.5\) (median \(16.6\)) \(\text{mg/dL}\). In 17 of 24 patients, bacterial infections were documented bacterially (4 Klebsiella pneumoniae, 3 Escherichia coli, and others.). There was no relationship between the level of G-CSF and WBC count, the grade of fever, the level of c-reactive protein, or the type of bacteria.

To examine any differences in G-CSF level among types of infection, we compared the G-CSF levels of patients with respiratory tract infection (RTI) and those of patients with urinary tract infection (UTI). The levels of G-CSF were \(734.2 \pm 822.0\) \(\text{pg/mL}\) and \(590.5 \pm 668.2\) \(\text{pg/mL}\), respectively, showing no significant difference (Table 2).

In 16 patients, the G-CSF levels in the acute stage of infection were compared with those after recovery phase (Fig 3). Fever and WBC in the acute phase were significantly higher than those after recovery phase (body temperature, \(38.4 \pm 1.0^\circ\text{C}\) versus \(36.5 \pm 0.3^\circ\text{C}\), \(P < .001\); WBC, \(13,806 \pm 5,067\) \(\mu\text{L}\) versus \(6,369 \pm 1,826\) \(\mu\text{L}\), \(P < .001\)). The level of the G-CSF in the acute phase was significantly higher than that after recovery phase (\(590.9 \pm 778.9\) \(\text{pg/mL}\) versus \(24.8 \pm 18.3\) \(\text{pg/mL}\)).

**Table 2. Serum G-CSF Levels, White Blood Cell (WBC) Counts and Fever in Respiratory Tract Infection (RTI) and Urinary Tract Infection (UTI)**

<table>
<thead>
<tr>
<th></th>
<th>Total Infection</th>
<th>RTI (n = 13)</th>
<th>UTI (n = 8)</th>
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<tbody>
<tr>
<td>G-CSF pg/mL</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>WBC/(\mu\text{L})</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fever (^\circ\text{C})</td>
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**DISCUSSION**

This study examined the serum G-CSF levels in patients with infections and in age-matched healthy volunteers. The range of serum G-CSF level in healthy volunteers (mean age: \(74.9 \pm 8.2\) years) was \(20 \text{ to } 95\) \(\text{pg/mL}\), and was less than \(60\) \(\text{pg/mL}\) in most cases. There was no difference in the level of G-CSF between males and females. This result was similar to that found by Watari et al2 in normal healthy volunteers (age 20 to 86, but mostly under age 60). As the method used for examination of serum G-CSF level was the same as that of Watari et al, results might indicate no significant difference in serum G-CSF levels based on age. The relationship between G-CSF level and WBC or granulocyte count was investigated in controls, but no significant relationship was found between them. This seems to be a natural result, because the WBC count is controlled by many factors besides G-CSF, such as cortisol, lymphokines, monokines, CFUs in bone marrow, spleen function and so on. We also examined the concentrations of serum G-CSF during one day in 3 normal healthy volunteers, and noted no significant change, although the level at midnight was not examined. Thus there is little possibility that the serum G-CSF level shows a diurnal rhythm.

In all cases of infection, the G-CSF level apparently increased in the acute phase, but was almost the same as that of controls after recovery phase. This suggests that G-CSF is directly or indirectly involved in reaction against infections. There were 5 patients whose serum G-CSF level was less than \(100\) \(\text{pg/mL}\) in the acute phase. All had a complication of old cerebral vascular disease for several years and repeated RTI and/or UTI. Patients with a history of repeated infection might have a low ability to produce G-CSF;
therefore it may be useful to monitor the serum G-CSF levels in patients with chronic or severe infections, and to administer recombinant G-CSF to them.

In bacterial infection, mononuclear cells affected by endotoxin are reported to produce G-CSF, IL-1 and TNF. On the other hand, soluble products from bacteria themselves, or products released from infected tissues through the action of bacteria induce G-CSF production in distant tissues. However, it is not clear which cells usually produce G-CSF in bacterial infection, ie stromal cells in bone marrow, or others such as endothelial cells.

It has been said that the levels of many cytokines are increased in infectious states. However, the only report of elevation of G-CSF levels in infectious states describes two from bacteria themselves, or products released from infected tissues. Another mechanism of G-CSF production is the release of certain cytokines from infected endothelial cells. These cytokines include IL-1 and TNF. IL-1 and TNF also induce fibroblasts and endothelial cells to produce G-CSF. In bacterial infection, mononuclear cells affected by endotoxin are reported to produce G-CSF, IL-1 and TNF also induce fibroblasts and endothelial cells to produce G-CSF. On the other hand, soluble products from bacteria themselves, or products released from infected tissues through the action of bacteria induce G-CSF production in distant tissues. However, it is not clear which cells usually produce G-CSF in bacterial infection, ie stromal cells in bone marrow, or others such as endothelial cells.

It has been said that the levels of many cytokines are increased in infectious states. However, the only report of elevation of G-CSF levels in infectious states describes two patients with AIDS-related infections. The present study is the first to investigate the serum levels of G-CSF in infected patients without hematologic disorders.

REFERENCES

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M Kawakami, H Tsutsumi, T Kumakawa, H Abe, M Hirai, S Kurosawa, M Mori and M Fukushima