血液中G-CSF濃度會影響受感染病人的復原力

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.

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.[1-6] 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. 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 complicating 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.[8] 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% polyethyleneglycol (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% H_2O_2) 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

Subjects	No.	G-CSF pg/mL
Total	32	25.3 ± 19.7
Male	8	25.1 ± 18.3
Female	24	25.3 ± 20.6
Over Age 75	14	28.8 ± 22.3
Under Age 75	18	22.6 ± 17.7

From the Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Tokyo, and Central Research Laboratories, Chugai Pharmaceutical Company, Tokyo, Japan.

Submitted April 13, 1990; accepted July 13, 1990.

Address reprint requests to Mutsumi Kawakami, MD, Department of Hematology, Tokyo Metropolitan Geriatric Hospital, Sakae-cho 35-2, Itabashi-ku, Tokyo 173, Japan.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" *in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

0006-4971/90/7610-0015$3.00/0

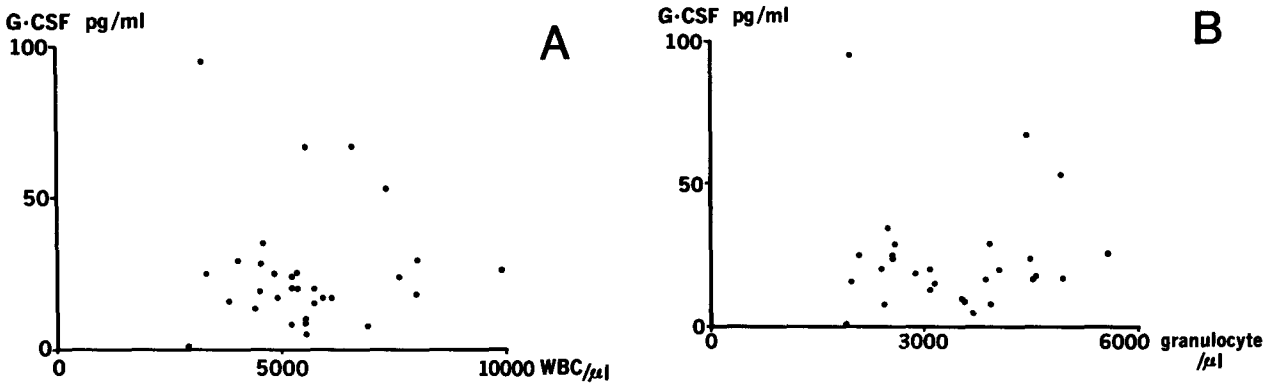

Fig 1. (A) Relationship between serum G-CSF level and peripheral white blood cell (WBC) count in elderly normal controls (n = 32). (B) Relationship between serum G-CSF level and blood granulocyte count in elderly normal controls (n = 28).

in the acute phase was 13,842.9 ± 4,644.3/L. 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 to 32.5 (median 16.6) 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 ± 822.0 pg/mL and 590.5 ± 668.2 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 ± 1.0°C versus 36.5 ± 0.3°C, $P < .001$; WBC, 13,806 ± 5,067/μL versus 6,369 ± 1,826/μL, $P < .001$). The level of the G-CSF in the acute phase was significantly higher than that after recovery phase (590.9 ± 778.9 pg/mL versus 24.8 ± 18.3 pg/mL).

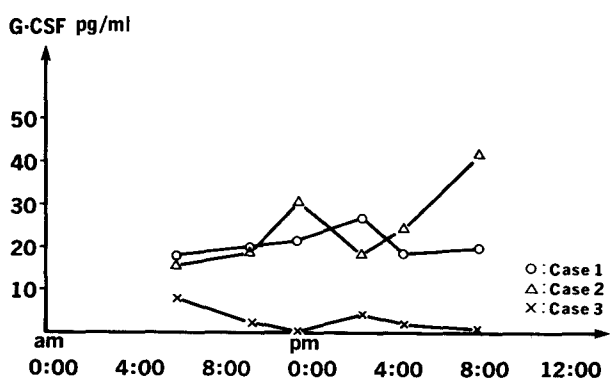

Fig 2. G-CSF concentrations during one day (n = 3). There was no significant diurnal change.

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 ± 8.2 years) was 20 to 95 pg/mL, and was less than 60 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 al[7] 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 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;

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

	Total Infection (n = 24)	RTI (n = 13)	UTI (n = 8)
G-CSF pg/mL	731.8 ± 895.0	734.2 ± 822.0	590.5 ± 668.2
WBC/μL	13,843 ± 4,644	14,550 ± 5,288	14,150 ± 3,517
Fever °C	38.6 ± 1.0	38.7 ± 0.8	38.8 ± 1.0

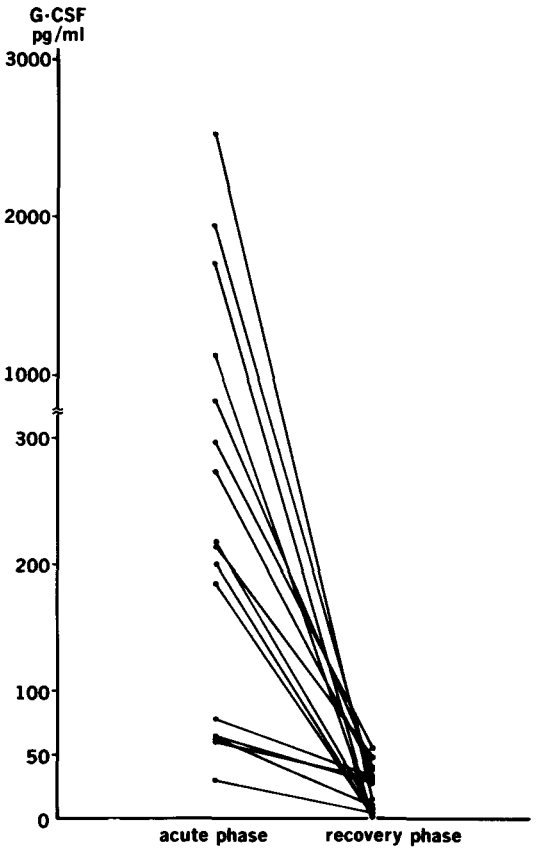

Fig 3. Serum G-CSF levels in acute phase of infection and after recovery phase (n = 16).

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.[9,10] IL-1 and TNF also induce fibroblasts and endothelial cells to produce G-CSF.[11-13] 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.[14-19] 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

1. Nicola NA, Metcalf D, Matsumoto M, Johnson GR: Purification of a factor inducing differentiation in murine myelomonocytic leukemia cells. J Biol Chem 258:9017, 1983

2. Platzer E, Welte K, Gabrilove J, Lu E, Harris P, Mertelsmann R, Moore MAS: Biological activities of human pluripotent hemopoietic colony stimulating factor on normal and leukemic cells. J Exp Med 162:1788, 1985

3. Welte K, Platzer E, Lu L, Gabrilove J, Levi E, Mertelsmann R, Moore MAS: Purification and biochemical characterization of human pluripotent hemapoietic colony stimulating factor. Proc Natl Acad Sci USA 82:1526, 1985

4. Souza LM, Boone TC, Gabrilove J, Lai PH, Zsebo KM, Murdock DC, Chazin VR, Bruszewsky J, Lu H, Chen KK, Barendt J, Platzer E, Moore MAS, Mertelsmann R, Welte K: Recombinant human granurocyte colony stimulating factor: Effects on normal and leukemic myeloid cells. Science 232:61, 1987

5. Nomura H, Imaezaki I, Oheda M, Kubota N, Tamura M, Ono M, Ueyama Y, Asano S: Purification and characterization of human granurocyte colony-stimulating factor (G-CSF). EMBO J 5:871,1986

6. Nagata S, Tsuchiya M, Asano S, Kaziro Y, Oheda M, Nomura H, Ono M: Molecular cloning and expression of cDNA for human granurocyte colony-stimulating factor. Nature 319:415,1987

7. Watari K, Asano S, Shirafuji N, Kodo H, Ozawa K, Takaku F, Kamachi S: Serum granulocyte colony-stimulating factor levels in healthy volunteers and patients with various disorders as estimated by enzyme immunoassay. Blood 73:117, 1989

8. Motojima H, Kobayashi T, Shimane M, Kamachi S, Fukushima M: Quantitative enzyme immunoassay for human granulocyte colony stimulating factor (G-CSF). J Immunol Method 118: 187,1989

9. Vellenga K, Rambaldi A, Ernst TJ, Ostapovicz D, Griffin JD: Independent regulation of M-CSF and G-CSF gene expression in human monocytes. Blood 71:1529, 1988

10. Ernst TJ, Ritchie AR, Demetri GD, Griffin JD: Regulation of granulocyte- and monocyte-colony stimulating factor mRNA levels in human blood monocytes is mediated primarily at a post-transcriptional levels. J Bio Chem 264:5700, 1989

11. Koeffler P, Gasson J, Ranyard J, Souza L, Shepard M, Munker R: Recombinant TNF stimulates production of granulocyte colony-stimulating factor. Blood 70:55, 1987

12. Seelentag WK, Mermod JJ, Montesano R, Vassalli P: Additive effects of interleukin 1 and tumor necrosis factor—on the accumulation of the three granulocyte and macrophage colony-stimulating factor mRNAs in human endotherial cells. EMBO J 6:2261, 1987

13. Zsebo KM, Yuschenkoff VN, Shiffer S, Chang D, MacCall E, Dinarello CA, Brown MA, Altrock B, Bagby GC: Vascular endotherial cells and granulopoiesis: Interleukin-1 stimulates release of G-CSF and GM-CSF. Blood 71:99, 1988

14. Bagby GC, Dinarello CA, Wallace P, Wagner C, Hefeneider S, MacCall E: Interleukin-1 stimulates granulocyte-macrophage colony-stimulating activity release by vascular endothelial cells. J Clin Invest 78:1316, 1986

15. Lovhaug D, Pelus LM, Nordlie EM, Boyum A, Moore MAS: Monocyte conditioned medium and macrophage colony-stimulating factor production in the adherent cell layer of murine bone marrow cultures. Exp Hematol NY 14:1037, 1986

16. Rennick D, Yang G, Gemmell L, Lee F: Control of hemopoiesis by a bone marrow stromal cell clone: Lipopolysaccharide— and interleukin-1—inducible production of colony-stimulating factors. Blood 69:682, 1987

17. Segal GM, MacCall E, Steve T, Bagby GC: Interleukin 1 stimulates endotherial cells to release multilineage human colony-stimulating activity. J Immunol 138:1772, 1987

18. Zucali JR, Dinarello CA, Oblon DJ, Gross MA, Anderson L, Weiner RS: Interleukin 1 stimulates fibroblasts to produce granulocyte-macrophage colony-stimulating activity and prostaglandin E2. J Clin Invest 77:1857, 1986

19. Cheers C, Haigh AM, Kelso A, Metcalf D, Stanley ER, Young AM: Production of colony-stimulating factors (CSFs) during infection: Separate determinations of macrophage-, granulocyte-macrophage-, and multi-CSFs. Infect Immun 56:247, 1988

1990 76: 1962-1964

Levels of serum granulocyte colony-stimulating factor in patients with infections

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

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.