The Level of Serum Granulocyte Colony-Stimulating Factor in Cancer Patients with Leukocytosis

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The level of serum granulocyte colony-stimulating factor (G-CSF) obtained from patients with leukocytosis ($>10,000/\mu\text{l}$) between May 1989 and April 1991 was measured by enzyme immunoassay. Studied were 18 patients with malignant neoplasms (median age, 64 years) and 14 patients with hematologic disease (median age, 59 years). Increased serum G-CSF values ranging from 70 to 374 pg/ml were noted in 7 of 15 lung cancer cases, a case of malignant thymoma and a blastic crisis of chronic myelogenous leukemia. The rest of the cases showed a normal value (<60 pg/ml). There was no correlation between the neutrophil count and G-CSF level. In lung cancer cases with high G-CSF values, neither a characteristic histologic type nor common elevation of tumor markers could be seen. The neutrophil alkaline phosphatase score was significantly increased and hypercalcemia was presented in high G-CSF cases. G-CSF may contribute at least in part to unknown leukocytosis observed in malignant neoplasms, especially in lung cancer.

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Key words: lung cancer, NAP, hypercalcemia

compared with hematologic diseases.

Introduction

The generation of granulocyte colony-stimulating factor (G-CSF) by monocytes, macrophages, fibroblasts and endothelial cells increases in response to infection (1) or neutropenia (2), via interactions of cytokines such as interleukin-1 (3) and tumor necrosis factor (1). Recently, recombinant human G-CSF has become available for clinical use and has contributed to the improvement of therapeutic results.

Leukocytosis is frequently noted among patients with cancer. A granulopoietic factor produced by malignant tumor was reported by Robinson (4), and Asano et al. (5) who demonstrated colony-stimulating activity in the plasma of patients with lung cancer provided evidence for the occurrence of G-CSF producing tumors. We previously reported G-CSF production by an established cell line derived from a patient with hepatoma (6).

In the present study, G-CSF levels in sera from patients with leukocytosis were examined and assessed to explore the potential contribution of circulating G-CSF in the development of leukocytosis in malignant neoplasms,

Subjects and Methods

Thirty-two patients with leukocytosis ($>10,000/\mu\text{l}$) between May 1989 and April 1991 were studied. They had no evidence of infection, nor influence of drugs at the time of the study. Of 18 patients with malignant neoplasms, the majority were lung cancer (15) and the remainder were a case each of malignant thymoma, malignant mesothelioma and prostatic cancer. There were 14 men and 4 women and their median age was 64 years (43 to 78 years). Of these, 14 patients (10 men, 4 women, median age 59; 38 to 72 years) had hematologic diseases; 3 patients with chronic myelogenous leukemia (CML, with blastic crisis in 2), 2 with chronic neutrophilic leukemia (CNL), 2 in the leukemic phase of chronic myelomonocytic leukemia (CMML), 2 with polycythemia vera, 1 with essential thrombocythemia and 4 with unclassified myeloproliferative disorder (MPD). Eight other cases of lung cancer without leukocytosis served as control.

The level of serum G-CSF was determined by enzyme

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immunoassay (7) at the Research Laboratories of Chugai Pharmaceutical Co. Ltd. Briefly, 0.2 ml of standards or unknowns were dispensed into the anti-recombinant G-CSF antibody-coated tubes followed by 0.5 ml of reaction buffer. After 2 hours of incubation at room temperature, horseradish peroxidase-labelled anti-recombinant G-CSF antibody (0.1 ml) was added and the tubes were further incubated for 2 hours at room temperature. The tubes were washed 3 times with washing buffer and 1 ml of substrate solution was added to each. After a 1 hour incubation at room temperature, the reaction was stopped with 1 ml of 4 N H₂SO₄ and the absorbance was measured at 492 nm. The G-CSF levels of healthy volunteers were less than 30 pg/ml (2). In routine measurement, the sensitivity of this assay was 60 pg/ml.

Sera were obtained from patients prior to institution of therapy as a rule. In cases where the patient had previously been or was being treated, blood was obtained at least one month after conclusion of the course of therapy for malignant neoplasms or during continued maintenance drug therapy for hematologic diseases.

Results

The serum G-CSF levels in patients with malignant neoplasms are shown in Table 1. Twelve of 18 cases were untreated at the time of study. None had evidence of overt infection but nearly all patients showed a positive test for C-reactive protein and 2 patients had a fever. Seven cases of lung cancer and one case of malignant thymoma showed high levels of serum G-CSF, with a range of 71 to 374 pg/ml (neutrophil count, 7,300 to 57,100/ μ l). The differential count of leukocytes revealed an overwhelming

predominance of mature neutrophils and a leukemoid reaction was seen in only 3 cases. The present series showed no correlation of neutrophil count with serum G-CSF level (correlation coefficient, 0.07, $0.5 < p < 1$). An increase in the monocyte count of over 1,000/ μ l was recognized in 3 patients among those with high serum G-CSF levels. There was no correlation between monocyte count and serum G-CSF level (correlation coefficient, 0.69, $0.05 < p < 0.1$). The serum level of G-CSF was under 60 pg/ml in all 8 lung cancer patients with a total leukocyte count of less than 10,000/ μ l (Table 2).

Table 3 shows serum G-CSF levels of patients with hematologic diseases. Ten of 14 cases, excluding CMMoL and blastic crisis of CML, were untreated at the time of study. Total leukocyte count exhibited a marked increase as high as 382,800/ μ l (neutrophil count, 7,400 to 49,800/ μ l) while the serum G-CSF level was less than 60 pg/ml in all cases except in one case of blastic crisis in CML which showed an elevation to 70 pg/ml.

Table 4 shows the histologic types and tumor markers in the 7 cases of lung cancer with high serum G-CSF levels. There was no characteristic histologic type in relation to the serum G-CSF elevation (adenocarcinoma, 2 cases; large cell carcinoma, 1; squamous cell carcinoma, 2; and unknown, 2). Serum assay data on tumor markers, which included squamous cell carcinoma related antigen (SCC), sialyl Le^x-i antigen (SLX), carcinoembryonic antigen (CEA), carbohydrate antigen (CA) 19-9, and neuron-specific enolase (NSE), also did not reveal any common elevation in these cases.

The neutrophil alkaline phosphatase (NAP) score and serum calcium level in 9 patients with high serum G-CSF values are shown in Table 5. The mean $\pm$ S.E. of the NAP score in lung cancer patients with a high G-CSF

Table 1. Serum G-CSF Level in Malignant Neoplasms with Leukocytosis

Patient	Disease	WBC / μ l	Neutro %			Mono %	CRP mg/dl	Fever	G-CSF pg/ml
			Immature	Stab	Seg				
KM 43 M	Lung ca	59,500	0	0	96	3	12.5	(+)	206
KA 47 M	Lung ca	57,100	0	1	94	3	16.6	(+)	72
YT 72 F	Lung ca	34,700	5	5	75	7	4.39	(-)	374
KT 50 F	Thymoma	27,500	2	0	96	1	5.57	(-)	81
TM 70 M	Lung ca	23,800	13	4	77	4	4.1	(-)	118
ST 63 F	Lung ca	18,500	0	0	74	7	24.4	(-)	<60
TI 78 M	Lung ca	16,600	0	1	94	4	1.12	(-)	166
NM 63 M	Lung ca	16,000	0	0	56	6	1.64	(-)	<60
KH 65 M	Lung ca	15,600	0	0	95	5	0.34	(-)	<60
MK 75 M	Mesothelioma	14,200	0	0	73	5	1.01	(-)	<60
MY 63 M	Lung ca	14,100	0	0	64	5	0.6	(-)	172
KI 66 M	Lung ca	14,100	0	0	52	5	2.4	(-)	71
RM 60 M	Lung ca	14,000	0	0	71	4	9.24	(-)	<60
HT 74 M	Lung ca	12,000	0	1	79	5	1.38	(-)	<60
TI 52 F	Lung ca	11,600	0	0	82	6	1.14	(-)	<60
SF 71 M	Lung ca	11,500	0	0	55	6	1.66	(-)	<60
KM 71 M	Lung ca	11,400	0	0	54	5	0.27	(-)	<60
YH 71 M	Prostatic ca	11,300	0	0	69	7	<0.25	(-)	<60

Serum G-CSF level in Cancer Patients

Table 2. Serum G-CSF Level in Lung Cancer Cases without Leukocytosis

Patient	Histology	WBC / μ l	Neutro %			Mono %	CRP mg/dl	Fever	G-CSF pg/ml
			Immature	Stab	Seg				
AK 64 M	Adeno + Squ.	9,800	0	0	66	3	1.03	(-)	<60
FY 61 M	Adeno	9,400	0	0	77	6	5.61	(-)	<60
KI 68 M	Large cell	8,200	0	0	71	7	1.8	(-)	<60
MY 48 M	Squamous	7,200	0	0	50	5	1.67	(-)	<60
SI 71 M	unknown	6,800	0	0	70	7	0.54	(-)	<60
SM 55 M	Squamous	6,600	0	2	51	5	<0.25	(-)	<60
MK 86 M	Adeno	6,200	0	0	66	3	<0.25	(-)	<60
HN 80 M	Squamous	6,000	0	0	48	8	<0.25	(-)	<60

Table 3. Serum G-CSF Level in Hematologic Diseases with Leukocytosis

Patient	Disease	WBC / μ l	Neutro %			Mono %	CRP mg/dl	Fever	G-CSF pg/ml
			Immature	Stab	Seg				
MT 65 M	CMMoL	382,800	86	6	7	0	0.75	(-)	<60
MO 59 F	CML crisis	93,400	74	3	26	0	6.38	(+)	<60
SK 42 F	CML crisis	81,000	30	0	19	0	3.74	(-)	70
SS 63 M	CMMoL	40,500	25	1	68	1	<0.25	(-)	<60
ST 57 M	CNL	39,300	0	3	72	2	<0.25	(-)	<60
SM 69 M	MPD	35,700	1	0	82	2	<0.25	(-)	<60
TO 72 M	Polycythemia	25,100	0	0	78	1	<0.25	(-)	<60
KM 69 M	Polycythemia	23,600	0	1	85	6	4.19	(-)	<60
SM 61 M	Thrombocythemia	19,500	0	0	51	8	<0.25	(-)	<60
TM 63 F	CNL	17,200	0	0	82	3	<0.25	(-)	<60
HT 38 M	CML	15,100	1	1	60	3	<0.25	(-)	<60
HS 69 F	MPD	13,800	0	2	64	0	<0.25	(-)	<60
IF 56 M	MPD	13,400	0	0	68	5	<0.25	(-)	<60
KH 47 M	MPD	12,700	0	0	58	2	<0.25	(-)	<60

Table 4. Histologic Types and Tumor Markers in Lung Cancer Cases with High Serum G-CSF Levels

Patient	Histology	Tumor marker				
		SCC (<1.5 ng/ml)	SLX (<38 U/ml)	CEA (<2.5 ng/ml)	CA 19-9 (<37 U/ml)	NSE (<10 ng/ml)
KM 43 M	Adeno	2.0	135.5	11.7	285.1	2.9
KA 47 M	Large cell	n.d.	n.d.	<1.0	<7.0	n.d.
YT 72 F	unknown	17.6	53.3	9.5	233.5	190.0
TM 70 M	Adeno	<1.0	29.1	n.d.	n.d.	26.0
TI 78 M	unknown	<1.0	33.0	1.3	19.3	16.0
MY 63 M	Squamous	35.0	29.6	3.7	21.8	8.0
KI 66 M	Squamous	15.0	18.4	<1.0	n.d.	6.7

n.d.: not done

level was 333.2 ± 33.5 ($n = 6$), which was significantly greater than that in those with serum G-CSF values of less than 60 pg/ml (265.4 ± 11.3 , $n = 14$) (Wilcoxon test $0.005 < p < 0.025$). Two patients with lung cancer and the patient with malignant thymoma had a high serum level of calcium in the present series.

Discussion

It is noteworthy that the incidence of serum G-CSF

elevation was as high as 47% in lung cancer with leukocytosis. This indicates that frequency for elevation of serum G-CSF was 6% in the total lung cancer cases (approximately 120 cases) examined during the two-year period of this study. A direct correlation of the neutrophil count and serum G-CSF level has been reported (2). Possible simultaneous elevation of M-CSF with G-CSF (8, 9) and/or possible participation of factors such as IL-3 and GM-CSF might account at least in part for the lack of a significant correlation of serum G-CSF

Table 5. NAP Score and Serum Calcium Level in Patient with High Serum G-CSF Values

Patient	Disease	G-CSF pg/ml	NAP		Ca mg/dl
			%	score	
YT 72 F	Lung ca	374	90	304	12.5
KM 43 M	Lung ca	206	n.d.	n.d.	11.5
MY 63 M	Lung ca	172	98	300	9.5
TI 78 M	Lung ca	166	92	312	8.6
TM 70 M	Lung ca	118	n.d.	n.d.	9.4
KT 50 F	Thymoma	81	100	344	14.0
KA 47 M	Lung ca	72	100	489	7.7
KI 66 M	Lung ca	71	83	250	10.1
SK 42 F	CML crisis	70	88	323	9.5

n.d.: not done

level and neutrophil count in the present series. The presence of an inhibitor of G-CSF may be probable (10) and CSF activity might be demonstrated by the transplantation to nude mice although it could not be detected in serum (11). Further study is needed, therefore, to demonstrate G-CSF in tumor tissues and to assess its biological activity in individual cases, in order to clarify the potential relationship of G-CSF to leukocytosis in malignant tumors.

On the other hand, the rarity of serum G-CSF elevation in hematologic diseases would suggest that G-CSF is unlikely to contribute in any significant extent to the pathogenesis of leukocytosis even in CNL which is a proliferative disorder of mature neutrophils. This may be explained by the feedback mechanism (2). It would be important to consider possible participation of plural hematopoietic factors (1, 3), such as the possibility of leukocytosis developing through cooperation of G-CSF and M-CSF (8). Furthermore, assessment of the sensitivity to CSF of myeloid precursor (CFU-C) is also important, from the viewpoints of 1) the reported findings of endogenous CFU-C proliferation in some cases of MPD (12) and 2) the fact that there is no significant correlation between serum G-CSF level and bone marrow CFU-C (13).

It was reported that large cell carcinoma is the most common histologic type of CSF-producing lung cancer (14). However, no characteristic features such as common histologic types or elevation of circulating tumor markers could be noted in the present series. The increase in NAP score seen in the cases exhibiting serum G-CSF elevation reflects promotion of neutrophil maturation by G-CSF (15) via its NAP-inducing effect (16, 17). Hypercalcemia in patients with G-CSF-producing carcinoma of the lung has been reported (18, 19), and it might be attributed to a PTH-like factor elaborated by the tumor (19, 20).

Unlike the leukocytosis in hematologic diseases, G-CSF may be implicated in the genesis of unknown leukocytosis in malignant neoplasms. The fact that

G-CSF receptor presents on tumor tissue of lung cancer (21) and G-CSF stimulates the growth of tumor cells (22, 23) favors the malignant tumor. G-CSF, on the other hand, has neutrophil function enhancing activities such as oxygen radical generation (24, 25), chemotaxis (26) and antibody-dependent cellular cytotoxicity (24), which all may afford an antitumor effect. The influence exerted by these inconsistent activities on the disease states is of profound interest and will be further investigated.

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