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Serum Concentrations of Granulocyte Colony-Stimulating Factor (G-CSF) Determined by A Highly-Sensitive Chemiluminescent Immunoassay during the Clinical Course of Subacute Thyroiditis

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Abstract. Granulocyte colony-stimulating factor (G-CSF) concentrations in serum were determined for the first time by a newly developed and highly sensitive chemiluminescent immunoassay (the limitation of detection, 0.5 pg/ml) in ten patients with subacute thyroiditis, during treatment with glucocorticoid or indomethacin. Before therapy, circulating neurophil counts significantly increased to $5.15 \pm 2.07 \times 10^3/\mu l$ compared with the convalescent phase ($2.94 \pm 1.07 \times 10^3/\mu l$), and the data were correlated with individual serum G-CSF levels ($r=0.854$, $P<0.01$). Serum concentrations of interleukin-3 (IL-3) and granulocyte-macrophage colony-stimulating factor (GM-CSF) were less than the detectable threshold of ELISA. During two weeks of glucocorticoid therapy, although the circulating neutrophil counts increased from $5.15 \pm 2.46 \times 10^3/\mu l$ to $7.73 \pm 1.64 \times 10^3/\mu l$ ($P<0.01$), serum G-CSF levels were depressed from 25.1 ± 15.3 pg/ml to 13.8 ± 13.9 pg/ml ($P<0.01$). These data indicate that G-CSF is one of the mediators of the increase of neutrophils in subacute thyroiditis, while it does not contribute to steroid-induced neutrophilia.

Key words: Granulocyte colony-stimulating factor (G-CSF), Chemiluminescent immunoassay, Subacute thyroiditis

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GRANULOCYTE colony-stimulating factor (G-CSF) is one of the hematopoietic cytokines which stimulate the proliferation and activation of neutrophils [1]. Recently human G-CSF was cloned and recombinant human G-CSF was introduced to be available for clinical application [2], but the pathophysiological role of endogeneous G-CSF has not been elucidated because the sensitivity of conventional methods including RIA and ELISA to measure G-CSF concentration has not been sufficient. More recently, we developed a highly-sensitive assay to determine G-CSF levels by chemiluminescent immunoassay [3], and it became possible to evaluate changes in G-CSF levels even within the normal range.

In this study, the relationship between circulating neutrophil counts and serum G-CSF concentrations was investigated in patients with subacute thyroiditis during treatment with glucocorticoid or indomethacin. Simultaneously, serum levels of other cytokines including interleukin-3 (IL-3) and granulocyte-macrophage colony stimulating factor (GM-CSF) which are hematopoietic cytokines for proliferation of neutrophils [4, 5], and interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) which are closely related to the inflammatory process [6, 7], were measured in the same

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sample. This is the first report which describes serum G-CSF concentration in subacute thyroiditis and changes in G-CSF levels during glucocorticoid therapy.

A Representative Case

Figure 1 shows an example of the clinical course of subacute thyroiditis. The patient was a 55-year-old woman, who came to the Kuma hospital with complaints of neck pain and palpitation. The circulating neutrophil count in peripheral blood was 4280/μl and CRP was strongly positive. The serum free T_4 level was high at 6.86 ng/dl while ^{123}I-radioactive iodine uptake was depressed. She was diagnosed as having subacute thyroiditis, and glucocorticoid therapy with dexamethasone (2 mg/day) was started. After two weeks of therapy, all symptoms had disappeared and the serum free T_4 level had recovered to within the normal range; then glucocorticoid therapy was discontinued after 6 weeks of therapy. As shown in Fig. 1, the circulating neutrophil count increased to 6440/μl at 2 weeks of therapy, and after cessation of treatment, the neutrophil count again decreased to 2000/μl which was below the pre-treatment level. The serum G-CSF level decreased from 19.0 pg/ml to 8.0 pg/ml at 2 weeks of therapy, reciprocally against the neutorphil counts. The final level of G-CSF after cessation of therapy was 12.0 pg/ml.

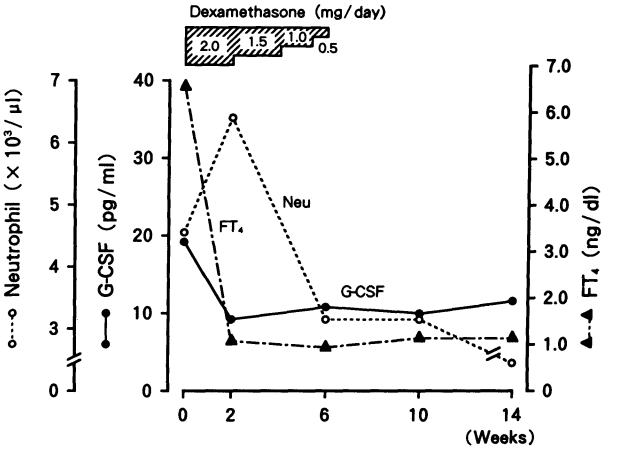

Fig. 1. Clinical course in a patient with subacute thyroiditis treated with dexamethasone. (●—●) denotes serum G-CSF concentrations; (○---○) circulating neutrophil counts; (▲—▲) serum free thyroxine (FT_4) concentrations.

Patients and Methods

Patients

Ten patients with subacute thyroiditis were studied. The patients' profiles and laboratory findings are summarized in Table 1. Thyroid-related autoantibodies including anti-thyroglobulin antibodies and antithyroid microsomal antibodies were all negative. Six of the patients were treated with glucocorticoids, starting with 2.0 mg/day of dexamethasone or betamethasone, and after 2 weeks of therapy the dosage was reduced and discontinued in 6 or 8 weeks. Four patients were treated with indomethacin (50 mg/day) for 4 or 6 weeks. The serum samples were stored at −30 °C before measurements of cytokines.

Table 1. Clinical characteristics of patients with subacute thyroiditis before therapy

Patient	Age (year)	Gender	WBC (/μl)	(Neu.) (/μl)	CRP	FreeT_4 (ng/dl)	TSH (mU/L)	Tg (ng/ml)	^{123}I-uptake (%/ 24h)	Therapy
1	55	F	6200	(4280)	5+	6.66	<0.10	613	0.9	dexamethasone
2	53	F	5800	(3940)	4+	2.14	<0.10	712	0.7	dexamethasone
3	43	F	3600	(2090)	3+	10.40	<0.10	1600	1.1	betamethasone
4	48	F	11600	(9400)	5+	8.36	<0.10	1600	1.1	betamethasone
5	51	F	7700	(5390)	4+	2.82	<0.10	584	1.1	betamethasone
6	48	M	8700	(5830)	5+	5.27	<0.10	ND	0.8	betamethasone
7	61	F	6700	(4290)	2+	2.59	<0.10	389	0.7	indomethacin
8	26	F	4500	(3200)	2+	3.67	<0.10	122	1.3	indomethacin
9	44	F	8700	(6440)	6+	2.45	<0.10	514	1.2	indomethacin
10	36	F	9100	(6640)	5+	2.18	<0.10	813	0.7	indomethacin

Neu., circulating neutrophil count; Tg, serum thyroglobulin; ND, not determined.

Determination of G-CSF levels

The serum G-CSF level was determined with a newly developed chemiluminescent immunoassay [3]. The sensitivity of this assay was 0.5 pg/m*l* and the measurable range in clinical application was from 1.0 to 1000 pg/m*l*. The intra-assay and inter-assay coeffeicients of variation were less than 5%. In normal subjects, the G-CSF concentrations were distributed from 6.2 to 31.9 pg/m*l* (n=33) with a mean of 15.9 ± 6.5 (SD) pg/m*l* and there were no significant daily variations in G-CSF levels [3]. In normal subjects, individual serum G-CSF levels did not correlate with circulating neutrophil counts. The serum samples for G-CSF measurement were stable for 6 months when stored at −30 °C.

Measurements of other cytokines and hormone levels

Serum concentrations of IL-3 and GM-CSF were determined with ELISA kits manufactured by Amersham International plc (UK), IL-1α and TNF-α by Ohtsuka Pharmaceutical Co. (Tokyo). Serum concentrations of free T_4, TSH and thyroglobulin were determined with commercially available RIA kits.

Circulating blood cell counts

Circulating blood cell counts were analyzed by the Technicon-H1 system (NY). The normal range of WBC was from 3.0 to 10.0 × $10^3/\mu l$; the normal distribution of circulating neutrophil counts was 40 to 75 percent of WBC.

Statistical analysis

Statistical significance was determined by paired Student's *t*-test and Wilcoxon rank sum test. A *P* value <0.05 was considered significant. Results are reported as the mean ± SD.

Results

Figure 2 shows changes in circulating neutrophil counts and serum G-CSF levels during treatment with glucocorticoid or indomethacin. Before treatment, the mean neutrophil count was 5.15 ± 2.07 × $10^3/\mu l$, and one month after cessation of treatment, neutrophils had significantly deceased to 2.94 ± 1.07 × $10^3/\mu l$ ($P<0.01$). The mean serum G-CSF levels before and after treatment were 21.8 ± 12.8 pg/m*l* and 15.3 ± 6.0 pg/m*l*, respectively. As shown in Fig. 3, a statistically significant correlation was found between individual neutrophil counts and serum G-CSF levels before therapy ($r=0.854$, $P<0.01$), but in all the samples serum IL-3 levels were less than 31.1 pg/m*l* which was the measurable threshold for the ELISA kit. Similarly GM-CSF levels were less than 7.8 pg/m*l* in all patients except one (15.0 pg/m*l*).

Figure 4 shows serial changes in neutrophil counts and G-CSF levels. Figure 4a shows the data for 4 patients treated with indomethacin. During 2 weeks of therapy, circulating neutrophil counts decreased from 5.14 ± 1.68 × $10^3/\mu l$ to 3.15 ± 0.84 ×

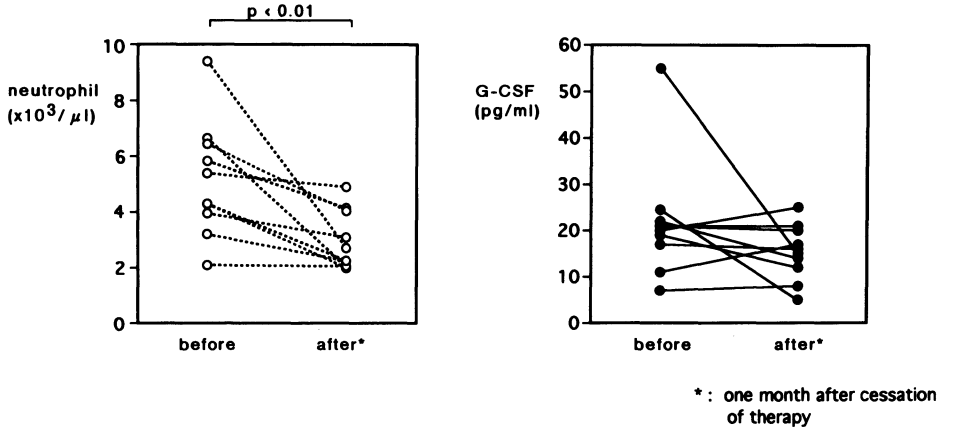

Fig. 2 Individual changes in circulating neutrophi counts (○---○) and serum G-CSF concentrations (●—●) in 10 patients with subacute thyroiditis during therapy.

$10^3/\mu l$ ($P<0.05$) and serum G-CSF levels from 16.8 ± 6.8 pg/ml to 12.3 ± 2.8 pg/ml. As shown in Fig. 4b, in 6 patients with glucocorticoid, the obvious increase in neutrophils was noted after 2 weeks of therapy; 5.15 ± 2.46 × $10^3/\mu l$ to 7.73 ± 1.64 × $10^3/\mu l$ ($P<0.01$), and again decreased to 3.14 ± 1.07 × $10^3/\mu l$, but G-CSF levels were significantly depressed after 2 weeks of glucocorticoid administration from 25.1 ± 15.3 pg/ml to 13.8 ± 13.9 pg/ml ($P<0.01$). After resolution of the disease, one month after cessation of therapy, serum G-CSF levels recovered to 17.2 ± 8.7 pg/ml.

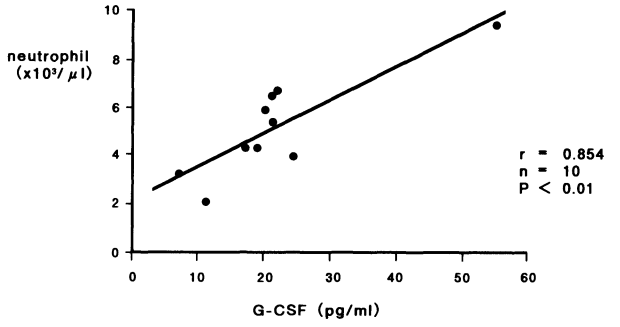

Fig. 3. Correlation between serum G-CSF concentrations and circulating neutrophil counts in patients with subacute thyroiditis before therapy.

Serum TNF-α concentrations during therapy are shown in Fig. 5. Only four samples among the 36 samples tested had a detectable value in ELISA, of which the detection threshold was 4.3 pg/ml. Therefore changes in TNF-α could not be evaluated. Although serum IL-1α concentrations were also measured in this study, all the samples were less than 7.8 pg/ml, which was the limit of detection in the conventional ELISA (data not shown).

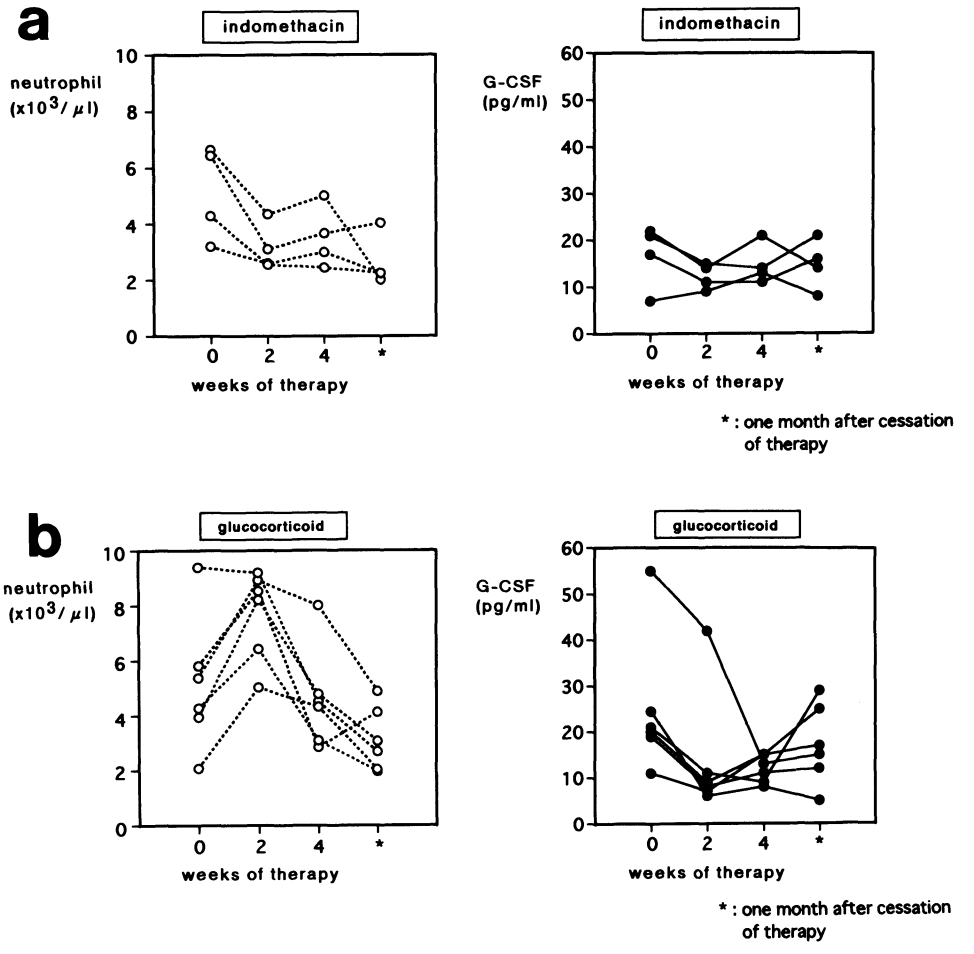

Fig. 4. Serial changes in circulating neutrophil counts (○---○) and serum G-CSF levels (●—●). Fig. 4a shows in patients treated with indomethacin, Fig. 4b shows those treated with glucocorticoid.

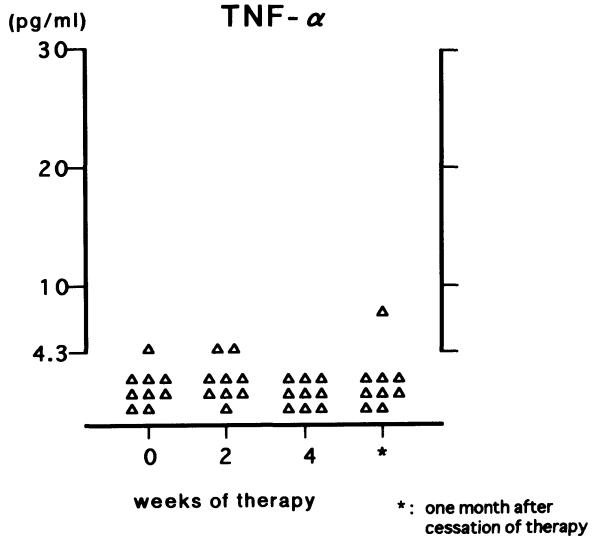

Fig. 5. Serum TNF-α concentrations in patients with subacute thyroditis during therapy. The detection threshold of this assay was 4.3 pg/ml.

Discussion

Subacute thyroiditis has been recognized as one of the viral infectious diseases [8], while recent reports suggest that autoimmunity may play a role in the etiology [9, 10]. Substantially some patients with subacute thyroiditis have thyroid related autoantibodies [11, 12], but in most of these cases transiently. It is suggested that patients with subacute thyroiditis may have immunological characteristics which induce an autoimmune reaction during the inflammatory process.

Although the circulating leukocyte count in patients with subacute thyroiditis is usually described as normal [8], a recent report stated that half of the patients had leukocytosis [13]. In the present study, circulating neutrophil counts in all patients were higher than after therapy. These data suggest that in subacute thyroiditis, some factors which contribute to the proliferation of neutrophils may exist during the active phase of the disease. Among several cytokines including IL-1, IL-3 and GM-CSF, which play a role in increase in neutrophils, G-CSF is a specific cytokine for granulocytosis [14]. Our newly developed chemiluminescent immunoassay for G-CSF is sensitive enough to investigate the changes in G-CSF levels even within the normal range [3]. In our data, although the serum G-CSF level was not significantly elevated compared with that of normal subjects, a positive correlation was observed between G-CSF levels and neutrophil counts before treatment, but such a correlation was not found in normal subjects. And in some patients a simultaneous decrease in neutrophils and G-CSF levels was observed from the acute to the convalescent phase. These results indicate that G-CSF is one of the cardinal mediators for circulating neutrophil counts in the active phase of subacute thyroiditis. Serum levels of IL-3 and GM-CSF could not be evaluated by conventional ELISA.

In most patients treated with glucocorticoid, circulating neutrophil counts increase during therapy, but the role of G-CSF is not known. In our results for subacute thyroiditis, neutrophil counts tended to be highest after 2 weeks of glucocorticoid therapy and then declined to low levels at resolution of the disease, but G-CSF levels were rather depressed during therapy (Fig. 4b). It is unclear whether the suppression of G-CSF reflected a direct effect of glucocorticoid *per se* or an indirect effect indicated by increased neutrophils. Anyway these results do not suggest that G-CSF contributes to the steroid-induced neutrophilia.

G-CSF increases in the active phase of the infection [15], together with other cytokines including IL-1 [6] and TNF [7], and accelerates the proliferation and migration of granulocytes. Presumably, in the resolution phase the G-CSF level may be suppressed according to the increase in circulating neutrophils. It has been reported that in the case of neutropenia, such as in patients with aplastic anemia or after bone marrow transplantation, serum G-CSF levels were quite high [14]. Recently, we reported a case of drug-induced agranulocytosis with spontaneous recovery, in which endogeneous G-CSF transiently increased before recovery of neutrophils [16]. These data suggest that the serum G-CSF level is regulated by something like a feedback mechanism similar to that in the regulation of pituitary hormones, *i.e.* ACTH which is stimulated by stress reaction and regulated by a negative feedback mechanism with cortisol.

In the present study, changes in serum levels of IL-1α and TNF-α, which are probably involved in the immunological reaction of subacute thyroiditis, were also investigated in the same samples as G-CSF. Recent studies have suggested that TNF-α not only plays a role in inflammation but may also

contribute to changes in thyroid hormone levels [17, 18]. However in the present study, the data obtained could not be evaluated because the sensitivity of conventional ELISA was not sufficient, as in the measurement of IL-3 and GM-CSF. New sensitive methods such as our G-CSF assay may be necessary to further access the role of other cytokines in the pathogenesis of subacute thyroiditis.

In conclusion, we have documented for the first time in this study the serum levels of G-CSF in patients with subacute thyroiditis by a highly sensitive assay, and demonstrated the significant depression of G-CSF levels during glucocorticoid therapy. Our findings indicate that G-CSF is one of the important mediators of the increase in circulating neutrophil counts in subacute thyroiditis.

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