Elevated Serum Granulocyte Colony-Stimulating Factor Levels in Patients With Active Phase of Sweet Syndrome and Patients With Active Behçet Disease

Implication in Neutrophil Apoptosis Dysfunction

Tamihiro Kawakami, MD, PhD; Syuichiro Ohashi, MD, PhD; Yoko Kawa, PhD; Hideto Takahama, MD, PhD; Masaru Ito, MD, PhD; Yoshinao Soma, MD, PhD; Masako Mizoguchi, MD, PhD

Background: Sweet syndrome (SS), an acute inflammatory disease, has clinical and laboratory features similar to those of Behçet disease (BD). Serum levels of granulocyte colony-stimulating factor (G-CSF) are elevated in patients with SS, and exogenous administration of G-CSF has repeatedly been implicated in the causation of SS. Granulocyte colony-stimulating factor is a hematopoietic growth factor that regulates the production and differentiation of neutrophils.

Objectives: To clarify the role of elevated serum G-CSF levels in patients with active SS and active BD compared with those with inactive SS or BD and healthy controls. To then analyze neutrophil apoptosis in the active state of SS and BD; and to also investigate the influence of autologous serum on neutrophil apoptosis.

Methods: Serum G-CSF was examined in 5 patients with active SS, 7 with inactive SS, 7 with active BD, 9 with inactive BD, and 5 healthy controls by means of an enzyme immunoassay kit. We measured apoptotic cells in the neutrophil fraction of peripheral blood collections in patients with active diseases and controls by means of flow cytometry.

Results: Serum G-CSF level was significantly higher in patients with active SS than in those with inactive SS. The difference in serum G-CSF levels among patients with active and inactive BD was also significant. Serum G-CSF level was significantly higher in patients with active SS than in those with active BD. Neutrophil apoptosis was significantly higher in patients with active SS than healthy controls. This increased apoptosis rate was also seen in patients with active BD. The increased rate of neutrophil apoptosis was significantly suppressed when the neutrophils were cultured for 18 hours in the presence of autologous active SS serum. Similarly, neutrophil apoptosis was suppressed in the presence of autologous serum in patients with active BD, but not significantly so.

Conclusions: These findings indicate that increased production of G-CSF in patients with SS and BD may play an important role in the manifestation of these disorders. Given the suppression of neutrophil apoptosis in the active state in the presence of the influence of autologous serum, which includes elevated G-CSF level, we propose that serum G-CSF plays a significant role in the suppression of neutrophil apoptosis. Furthermore, G-CSF–induced suppression of neutrophil apoptosis appears to be deeply involved in the pathogenesis of SS and BD.

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more, our group previously suggested that G-CSF is intensively involved in BD.13

Granulocyte colony-stimulating factor is a hematopoietic growth factor that increases the number of peripheral neutrophils and active mature neutrophils.14,15 In steady-state hematopoiesis, G-CSF regulates the level of neutrophils in bone marrow and blood and is critical for the survival of granulocytic cells. The factor strongly influences neutrophil function by stimulating production, activation, maturation, and chemotaxis of granulocytes.16 Philpott et al17 showed that G-CSF suppressed apoptosis in vivo in a CD34+ cell population and thereby prolonged their survival.

In the present study, we attempt to measure serum G-CSF concentrations in patients with SS and BD and to evaluate the significance of disease activity. We subsequently examine the abnormal neutrophil apoptosis mechanism attributed to neutrophils in the active state of SS or BD.

METHODS

CLINICAL INVESTIGATION

The medical records of patients coded with the diagnosis of SS or BD were reviewed in a dermatology practice that examines university-based patients. Twelve cases satisfied the diagnostic criteria for SS.18 Sixteen patients presented with BD, diagnosed according to the criteria proposed by the International Study Group for Behc¸et’s Disease.19,20 All patients were further divided according to either active or inactive state of the disease. Both clinical and laboratory findings were used for the diagnosis of active and inactive SS or BD. Active state was defined by worsening of clinical SS symptoms at the time of study, and the presence of at least both major criteria (abrupt onset setting by the same operator.

Figure 1

STATISTICAL ANALYSIS

The 2-tailed, unpaired t test was used to compare statistical differences between 2 groups. The level of significance was set at \( P < .05 \) in all cases.

RESULTS

MEASUREMENT OF G-CSF IN SERUM

The mean serum G-CSF concentration was significantly higher (\( P < .01 \)) in patients with active SS (115.4 ± 33.2 pmol/L) vs patients with inactive SS (Figure 1). Patients with BD in the active stage had significantly (\( P = .002 \)) higher serum G-CSF concentrations (34.1 ± 12.8 pmol/L) than pa-
COMMENT

The findings from this study demonstrated that G-CSF levels in peripheral blood are significantly higher in active SS and active BD vs the inactive stage. The G-CSF is a growth factor that promotes the production and maturation of myeloid cells and, in particular, the proliferation and differentiation of neutrophil progenitors both in vitro and in vivo. Treatment with G-CSF can exacerbate preexisting inflammatory conditions, presumably by stimulating neutrophil proliferation and activity at sites of chronic inflammation. High levels of G-CSF in active SS or BD seem to be a useful indicator of the

The percentage of apoptotic nuclei in neutrophils from patients with active Sweet syndrome after neutrophils were suspended in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY) or 10% FCS plus autologous serum incubated for 0, 3, 6, or 18 hours. Autologous serum treatment induced a marked reduction in the proportion of neutrophil apoptosis after incubation for 18 hours.

To better understand apoptosis of neutrophils in the active disease state, we incubated these cells in medium supplemented with 10% FCS and added 20% autologous serum. The solution was then analyzed by flow cytometry. Under these conditions, the rate of apoptosis of neutrophils in patients with active SS was significantly attenuated at 18 hours (P<.05; Figure 3). Autologous serum induced a marked reduction in the percentage of hypodiploid forms, which are characteristic of apoptotic nuclei, and there was a concomitant increase in the proportion of diploid nuclei. This indicates that autologous serum induces a remarkable inhibition of neutrophil apoptosis in patients with active SS.

The same methods were applied to neutrophils from patients in the active stage of BD. All assays were performed with the use of culture medium supplemented with 10% FCS and 20% autologous serum. To analyze whether proteolytic fragments generated by the action of autologous serum could be involved in the stimulation of neutrophil apoptosis, cells were incubated in 10% FCS or 10% FCS plus BD autologous serum for 0, 3, 6, and 18 hours. There was a distinct attenuation of apoptosis among nuclei treated with autologous serum for 18 hours vs those that were not (Figure 4).

The percentage of apoptotic nuclei (reduced DNA content due to fragmentation and enhanced chromatin condensation) in neutrophils from patients with active Sweet syndrome (SS), inactive SS, active Behc¸et disease (BD), and inactive BD and healthy controls.

Figure 1. Serum granulocyte colony-stimulating factor (G-CSF) levels in patients with active Sweet syndrome (SS), inactive SS, active Behc¸et disease (BD), and inactive BD and healthy controls.

Figure 2. Percentage of apoptotic nuclei (reduced DNA content due to fragmentation and enhanced chromatin condensation) in neutrophils from patients with active Sweet syndrome, patients with active Behc¸et disease, and healthy controls, augmented in cell culture. The percentage of apoptotic neutrophils in patients with active Sweet syndrome increased significantly after 18 hours of incubation compared with controls.

Figure 3. Percentage of apoptotic nuclei in neutrophils from patients with active Sweet syndrome after neutrophils were suspended in RPMI 1640 supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY) or 10% FCS plus autologous serum incubated for 0, 3, 6, or 18 hours.

Figure 4.
activity level in these diseases. Consequently, we propose that G-CSF may induce the active phase of SS via stimulatory effects on the production and function of neutrophils. The molecular basis for enhanced neutrophil function in the active state is unknown. Elevated serum G-CSF levels suggest that the cytokine physiologic activity of this compound may be involved. This hypothesis is supported in part by the present results concerning G-CSF. We further speculated that elevated G-CSF concentrations in inflammatory states may produce the associated neutrophilia. The maintenance of neutrophil numbers seen in active SS and BD is an important feature in response to inflammatory stimuli. Neutrophil chemotaxis has been found to be enhanced, and this has been attributed to the increased level of a chemoattractant in the serum.

Both BD and SS yield similar laboratory findings, but the clinical course of SS is different from that of BD. In SS, most symptoms appear at the same time, while 1 or more symptoms of BD appear in the acute phase. After the initial symptoms appear, it takes several years for other symptoms to occur in BD, which is a chronic inflammatory disease with remissions and relapses. The patients with active SS had significantly higher serum G-CSF levels than the patients with active BD. This finding suggests that increased G-CSF serum levels play a prominent role in the worsening of SS, and at least some part in the worsening of BD. The elevated G-CSF levels in active BD may play a direct role in the disease process. Although we have documented quantitative G-CSF abnormalities in patients with active SS or BD, the difference in cause between SS and BD, including the role of G-CSF, remains unclear.

An inverse relationship between serum G-CSF level and active phase in our patients suggests a physiologic role of G-CSF in neutrophil homeostasis. The G-CSF prolongs survival of neutrophils while enhancing chemotaxis, phagocytosis, superoxide generation, and other metabolic functions. We evaluated the correlation between serum G-CSF level and neutrophil apoptosis in patients with active SS, those with active BD, and healthy controls by means of flow cytometry. Apoptosis, or programmed cell death, is a critical process in the regulation of cellular proliferation and differentiation. In normal hematopoiesis, apoptosis is thought to be involved in regulating the rate of committed cell production. It is responsible for the deletion of unwanted cells in different processes, including inflammation. Neutrophil apoptosis can be modulated by a variety of inflammatory mediators. Using culture medium supplemented with 10% FCS, we analyzed apoptotic neutrophil proportion. An accelerated rate of apoptosis was noted at 3, 6, and 18 hours of incubation in patients with active SS and active BD compared with healthy controls, and the percentage of apoptotic nuclei was significantly higher among active SS neutrophils at 18 hours compared with those of healthy controls. Similarly, the percentage of apoptotic neutrophils in active BD was higher than that in controls. This indicates that neutrophil apoptosis is accelerated in the active phase of disease. Furthermore, these data, based on incubation in FCS medium alone, suggest that apoptosis plays a large role in dramatically reducing neutrophilia in the active phase.

The inclusion of autologous serum led to a marked reduction in the rate of neutrophil apoptosis, and this was most prominent at 18 hours. Adding autologous serum to active SS or BD neutrophils was a more potent inhibitor of neutrophil apoptosis than FCS alone, suggesting a specific role for G-CSF in the regulation of neutrophil homeostasis. Apoptosis has been explained by a variety of neutrophil functional defects. The neutrophil apoptosis system seems to become less effective with progression of disease activity. This suggests that dysfunction associated with neutrophil apoptosis is an early, biologically relevant risk factor for active progression of the disease. The possible suppression of apoptosis in abnormal neutrophils by G-CSF requires further investigation. Furthermore, by better understanding the pathogenesis of this disorder, it may be possible to develop more effective treatment strategies for SS and BD through the induction of neutrophil apoptosis.

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Corresponding author and reprints: Tamihiro Kawakami, MD, Department of Dermatology, St Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki, Kanagawa, 216-8511, Japan (e-mail: tami@marianma-u.ac.jp).

REFERENCES


Figure 4. Percentage of apoptotic nuclei in neutrophils from patients with active Behçet disease after neutrophils were treated with 10% fetal calf serum (FCS) (GIBCO, Grand Island, NY) or 10% FCS plus autologous serum. Analysis of apoptosis by flow cytometry, performed after incubation for 3, 6, and 18 hours, reduced by autologous serum treatment.
The First International Conference on Cutaneous Lupus Erythematosus will be held from September 1 to September 4, 2004, in Duesseldorf, Germany, in cooperation with the American College of Rheumatology (ACR) Response Criteria Committee on SLE. Abstract submission deadline is June 15, 2004.

Organizing the conference are the Departments of Dermatology and Rheumatology, University of Duesseldorf; Department of Dermatology, University of Witten-Herdecke, Germany; and the Department of Dermatology, University of Iowa College of Medicine. The organizers are A. Kuhn, T. Ruzicka, and M. Schneider, Duesseldorf, Germany, and R.D. Sontheimer from Iowa.

For more information, see our Web site at http://www.CLE2004.rheumanet.org, or contact Annegret Kuhn, MD, University of Duesseldorf, Moorstrasse 5, D-40225 Duesseldorf, Germany (phone: ++49 (0) 2118117600 or 8798; fax: ++49 (0) 2118119175; e-mail: LECONGRESS2004@uni-duesseldorf.de).