I. INTRODUCTION

Human granulocyte colony-stimulating factor (G-CSF) is a glycoprotein (MW 19,000) which stimulates the production and functional activation of neutrophils. G-CSF is supposed to play an important role as a circulating hematopoietic factor regulating the peripheral blood neutrophil level according to the pathological situation. Serum G-CSF levels are reactively elevated in various hematological and non-hematological disorders. Conversely, G-CSF itself may be involved in the pathogenesis under some circumstances. Here we discuss the biological aspects of G-CSF and its pathophysiological roles.

II. BIOLOGICAL ACTIVITIES OF G-CSF

G-CSF stimulates the proliferation and differentiation of neutrophilic progenitor cells in vitro. Similarly, daily administration of rhG-CSF to laboratory animals and human volunteers stimulates the proliferation, differentiation, and maturation of neutrophilic cells at various stages of differentiation, resulting in marked myeloid hyperplasia in bone marrow and substantial peripheral neutrophilia.

It was reported that G-CSF enhances interleukin 3-dependent proliferation of murine multipotential hematopoietic progenitor cells in vitro [1], suggesting that G-CSF also acts on relatively primitive hematopoietic stem cells. Actually stem and progenitor cell pool in bone marrow increases in size during rhG-CSF administration.

G-CSF stimulates the survival and adhesion of mature neutrophils and activates their function (superoxide production, migration, phagocytosis, ADCC activity, etc.). Moreover, G-CSF enhances the release of these mature neutrophils and stem/progenitor cells from bone marrow.

In addition, G-CSF stimulates the proliferation of myeloid leukemia cells.

Myeloid marker gene expression in bone marrow cells was influenced by G-CSF treatment (Tsuruta, T. et al., manuscript in preparation). For example, myeloperoxidase (early myeloid marker) mRNA expression by bone marrow mononuclear cells was increased after G-CSF stimulation. The expression of neutrophil alkaline phosphatase (NAP, late myeloid marker) mRNA was markedly increased by G-CSF treatment both in mononuclear and polymorphonuclear fractions of bone marrow. Interestingly, GM-CSF inhibited not only the spontaneous NAP mRNA expression but also the G-CSF-induced NAP mRNA expression, suggesting the different physiological roles of G-CSF and GM-CSF in neutrophilic maturation.

III. REGULATORY PRODUCTION OF G-CSF

A variety of cells are known to produce G-CSF in vitro upon stimulation. Lipopolysaccharide (LPS) stimulates monocytes/macrophages and stromal cells
to produce G-CSF. Cytokines secreted from activated T cells such as IL-3, IL-4, GM-CSF, and interferon(IFN)-γ also stimulate monocytes/macrophages to produce G-CSF. These activated monocytes/macrophages also secrete IL-1 and TNFα, both of which stimulate fibroblasts, endothelial cells, and stromal cells to produce G-CSF. This complicated network participates in the regulation of G-CSF production in inflammation.

Bone marrow stromal cells may be important as physiological G-CSF-producing cells, because these cells are believed to be in contact with hematopoietic stem cells and are crucial to the maintenance of hematopoiesis. Stromal cells are composed of heterogeneous cell populations, including fibroblasts, preadipocytes, adipocytes, monocytes/macrophages, and endothelial cells. When G-CSF production by primary human bone marrow stromal cells was examined using in situ hybridization, only a small proportion of the IL-1- or LPS-treated stromal cells exhibited strong positive reaction (Watari, K. et al., manuscript in preparation). Therefore, stromal cells are heterogeneous in terms of the capacity for G-CSF production.

Besides the inflammatory or reactive G-CSF production induced by LFB, IL-1, or TNFα, bone marrow stromal cells may produce a small amount of G-CSF constitutively. Interaction between immature myeloid cells and stromal cells may induce G-CSF production. To explore this possibility, stromal cells were cocultured with various myeloid cell lines and G-CSF mRNA expression was examined by Northern analysis. An IL-3/G-CSF-dependent murine myeloid leukemia cell line (NFS-60) was cultured on PA6 murine stromal cell line, which is a preadipocyte cell line with the ability to support hematopoiesis. NFS-60 cells strongly adhered to PA6 stromal cells during coculture. Interestingly, not only LPS/TPA stimulation but also coculture with NFS-60 cells apparently induced G-CSF mRNA expression (Yoshikubo, T. et al., manuscript in preparation). Direct cell-to-cell interaction was crucial to this phenomenon. Further study will be required to determine whether this kind of mechanism physiologically acts to produce a small amount of G-CSF for the maintenance of basal-level myelopoiesis in bone marrow.

IV. SERUM G-CSF LEVELS IN PATIENTS WITH VARIOUS DISORDERS

The measurement of serum G-CSF level by enzyme immunoassay (EIA) provided valuable information as to the pathophysiological role of G-CSF [2]. The serum G-CSF levels in healthy volunteers were almost undetectable (<30pg/ml). Figure 1 presents the data of serum G-CSF levels in three representative disorders. In aplastic anemia, there was an inverse correlation between blood neutrophil count and serum G-CSF level. When the serum G-CSF levels were estimated before and after allogeneic bone marrow transplantation (BMT) in a patient with severe aplastic anemia, the levels decreased along with neutrophilic recovery. Moreover, serum G-CSF levels were elevated during neutropenic period in a patient with cyclic neutropenia. These findings suggest that the serum G-CSF level is regulated by a feedback mechanism.

Transient elevation of serum G-CSF level was also observed after the conditioning for BMT or anti-cancer chemotherapy. This kind of increase may have some relation to tissue damage induced by chemoradiotherapy, in addition to feedback mechanism induced by severe neutropenia.

On the other hand, some patients with infectious disorders and lung cancer showed high serum G-CSF levels associated with increased blood neutrophil count, presumably reflecting reactive and aberrant production of G-CSF, respectively.

These observations indicate that G-CSF production is usually regulated to maintain a constant peripheral blood neutrophil level in the healthy state
and to elevate the level to cope with pathological situations such as microbial infections.

V. G-CSF AND G-CSF RECEPTOR mRNA EXPRESSION BY MYELOID LEUKEMIA CELLS

Northern analysis showed that myeloid leukemia cells in most cases expressed G-CSF receptor mRNA. This finding is compatible with the presence of G-CSF-binding sites on these cells and their responsiveness to G-CSF in culture. Therefore, the growth of myeloid leukemia cells in vivo may be enhanced in response to endogenous G-CSF produced by stromal cells (paracrine growth). On the other hand, myeloid leukemia cells in some cases are known to produce G-CSF, especially in monocytic leukemia such as M4 and M5 acute myeloid leukemia. If both G-CSF and G-CSF receptor are expressed in the same myeloid leukemia cells, autocrine growth will occur. In this regard, it is worthy of note that the spontaneous leukemic colony formation was frequently observed without the addition of CSF in leukemic progenitor assay mainly in cases of M4 and M5. The levels of G-CSF and G-CSF receptor mRNA expression by myeloid leukemia cells may influence their growth characteristics and clinical features.

VI. DECREASED PRODUCTION OF G-CSF BY STROMAL CELLS IN BONE MARROW FAILURE

As described above, bone marrow stromal cells have the ability to produce G-CSF. The G-CSF-producing capacity of primary cultured stromal cells was examined in normal volunteers and various hematological disorders.

![Figure 1. Relationship between serum G-CSF level and blood neutrophil count in patients with aplastic anemia, infectious disorders, or lung cancer [2].](image)

Absolute neutrophil count is indicated on the abscissa. The serum G-CSF level estimated by EIA is shown on the ordinate. A reverse correlation between blood neutrophil count and serum G-CSF level was observed for aplastic anemia (r=-0.8169, p<0.01).
Culture supernatants were harvested after IL-1 or LPS stimulation of stromal cells, and the G-CSF content was determined by EIA. As a result, G-CSF production was severely depressed in two of seven cases of aplastic anemia [3]. Although the pathogenesis of aplastic anemia is heterogeneous and variable among patients, our results suggest that reduced ability of stromal cells to produce G-CSF might partly explain the hematopoietic suppression observed in some cases of aplastic anemia. In relation to this phenomenon, there was an interesting report that cytomegalovirus (CMV) infection of stromal cells reduced their G-CSF production [4]. This may be one of the reasons for neutropenia observed in CMV infection. Further study will be required to elucidate the involvement of decreased cytokine production in various types of bone marrow failure.

SUMMARY

G-CSF is produced by a variety of cells. In situ hybridization showed that only a small proportion of stromal cells expressed G-CSF after stimulation with LPS or IL-1. The measurement of serum G-CSF level by enzyme immunoassay provided valuable information as to the pathophysiological roles of G-CSF. In aplastic anemia, there was an inverse correlation between blood neutrophil count and serum G-CSF level. Similarly, the G-CSF level rose during the neutropenic phase of cyclic neutropenia. These findings suggest that the serum G-CSF level is regulated by a feedback mechanism. In some cases, the reduced G-CSF production by stromal cells may underlie the pathogenesis of neutropenia. On the other hand, infections and cancers sometimes caused high serum G-CSF levels in association with increased blood neutrophils, presumably reflecting reactive and aberrant production of G-CSF, respectively. Expression of G-CSF by myeloid leukemia cells may partly contribute to their abnormal growth through the autocrine mechanism.

REFERENCES


