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THE IMPORTANCE OF THE GRANULOCYTE-COLONY STIMULATING FACTOR IN ONCOLOGY

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Abstract

Granulocyte-colony stimulating factor (G-CSF) is a glycoprotein, the second CSF, sharing some common effects with granulocyte macrophage-colony stimulating factor (GM-CSF), interleukin-3 (IL-3) and interleukin-5 (IL-5). G-CSF is mainly produced by fibroblasts and endothelial cells from bone marrow stroma and by immunocompetent cells (monocytes, macrophages). The receptor for G-CSF (G-CSFR) is part of the cytokine and hematopoietin receptor superfamily and G-CSFR mutations cause severe congenital neutropenia.

The main action of G-CSF - G-CSFR linkage is stimulation of the production, mobilization, survival and chemotaxis of neutrophils, but there are many other G-CSF effects: growth and migration of endothelial cells, decrease of norepinephrine reuptake, increase in osteoclastic activity and decrease in osteoblast activity.

In oncology, G-CSF is utilized especially for the primary prophylaxis of chemotherapy-induced neutropenia, but it can be used for hematopoietic stem cell transplantation, it can produce monocytic differentiation of some myeloid leukemias and it can increase some drug resistance.

The therapeutic indications of G-CSF are becoming more and more numerous: non neutropenic patients infections, reproductive medicine, neurological disturbances, regeneration therapy after acute myocardial infarction and of skeletal muscle, and hepatitis C therapy.

Keywords: G-CSF, G-CSFR, neutropenia, therapeutic indications

G-CSF structure and synthesis

Granulocyte-colony stimulating factor (G-CSF) and its related granulocyte macrophage-colony stimulating factor (GM-CSF) are glycoproteins belonging to the family of colony-stimulating factors (CSF) [1]. The CSF family includes CSF-1 or macrophage CSF (M-CSF), CSF-2 or granulocyte macrophage CSF (GM-CSF), and CSF-3 or

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granulocyte CSF (G-CSF) [2].

G-CSF, GM-CSF and erythropoietin (EPO) are also hematopoietic cytokines [3]. GM-CSF, interleukin-3 (IL-3) and interleukin-5 (IL-5) are members of the same cytokine's family that share a beta receptor subunit (βc) [4] and G-CSF exhibits synergistic activity with IL-3 [5].

G-CSF and GM-CSF are ubiquitous growth factors, found in a wide variety of tissue types, including reproduction organs [6], but they are mainly produced by resting or stimulated stromal cells of the hematopoietic microenvironment (fibroblasts and endothelial cells) and by immunocompetent cells (monocytes, macrophages) [7]. In addition, GM-CSF is produced by T lymphocytes and smooth muscle cells [8,9]. The exposure to bacterial lipopolysaccharides (LPSs) results in the activation of inflammatory respons of the host to invading patogen. Due to this activation, the production of both growth factors by monocytes and macrophages is triggered [10,11].

This cytokine regulates the growth, differentiation, migration and effector function activities of many hematopoietic cells in bone marrow, blood and sites of inflammation [4].

In terms of tolerance and mobilization of hematopoietic progenitor cells (CD34+ cells) and leukocytes, G-CSF is superior to GM-CSF [12].

Regarding dendritic cells (DC) activation, GM-CSF seems to preferentially enhance the number and activity of type 1 dendritic cells (DC1). The DC are responsible for the activation of the immune response under tissue malignant transformation, injury or infection. DC1 are myeloid dendritic cells, they play an important role in turning T-cells toward Th-1 phenotype with anti-tumor effects. All these sustain the role of GM-CSF as an immune stimulant in cancer patients [13].

G-CSF signaling and receptor

The diverse biological effects are mediated through the interaction of G-CSF with a cell-surface receptor expressed on responsive cells [14]. Therefore the expression of the receptor on myeloid progenitors plays a crucial role for the G-CSF induced myelopoiesis [15].

The receptor for G-CSF (G-CSFR) is part of the cytokine receptor superfamily [5] and hematopoietin receptor superfamily [16] and was detected in a variety of hemopoietic cells [17].

G-CSF binding to G-CSFR induces intracellular protein tyrosine phosphorylation and triggers multiple signaling mechanisms: activation of JAK tyrosine kinases and signal transducers and activators of transcription (STAT) proteins, activation of the ras-MAP kinase route, processes that results in induction of gene transcription [18].

G-CSF induces the mobilization of hematopoietic stem cells (HSC) from the bone after splitting the links between them and niche where they are stored, connections made by certain retention factors: vascular cell adhesion molecule 1 (VCAM-1)/integrin α4β1 (very late antigen-4, VLA-4) and the chemokine CXCL12 (stromal cell-derived factor-1, SDF-1)/CXCR-4 receptor. These connections may be split by the CXCR-4 antagonists, which block the CXCL12 retention activity and mobilize HSC (e.g., the compound AMD3100 mobilizes HSC at about 9 hours after administration) and by the VLA-4 antagonists, which block the favor the VCAM-1 retention activity and mobilize HSC (e.g., the compound BIO5192). The protease enzyme necessary for HSC mobilization is the amino-dipeptidase CD26 [19]. Others mechanisms of G-CSF action in the bone marrow for HSC mobilization implies the chemokine stromal cell derived factor 1 (SDF-1) and its receptor CXCR4. When neutralizing the CXCR4 or SDF-1 antibodies the stem cells' mobilization was observed [20].

Patients with hypomorphic mutations at G-CSFR exhibit marked neutropenia, whereas in cases where the genetic mutation causes hyperactivation of this receptor, neutrophilia is observed [21]. In cases with severe congenital neutropenia, the G-CSFR mutations might be involved in leukemogenesis [18].

G-CSF effects

The role of the G-CSFs in the synthesis of granulocytes

Neutrophils, eosinophils, and basophils are part of the granulocyte family which plays an essential role in the immune system. They are activated during microbe-induced and sterile inflammation. The factors that regulate the survival and death of granulocytes controls (in part) the severity of this type of inflammatory processes [22].

The principal action of G-CSF is expressed upon the neutrophils, having a role in both stimulation of hematopoietic progenitors, and production and mobilization of neutrophils from the bone marrow, as well as in the survival of mature neutrophils [19].

Also G-CSF influences the survival and chemotaxis of neutrophils [23]. Acting on neutrophils, G-CSF stimulates the release of arachidonic acid, the production of leukocyte alkaline phosphatase (LAP), myeloperoxidase and superoxide anion [24].

It was noted that G-CSF is essential in the production of an increased number of neutrophils in case of infections. However, studies in G-CSF genetic deficient mice showed that the formation of granulocytes under conditions of homeostasis was possible in a proportion of 25%. The rate of production of neutrophils is regulated to a large extent by the degree of their apoptosis in tissues [25].

Other G-CSF effects

G-CSF can exert effects on cells outside the granulocyte lineage [26].

G-CSF and GM-CSF exert similar effects. Following local and systemic GM-CSF administration support, it was shown that the immune stimulant effect in cancer patients

of GM-CSF is due to the preferentially enhancement of both the numbers and activity of DC1, responsible for initiating cytotoxic immune responses [13].

Administration of G-CSF can produce *in vitro* proliferation or monocytic differentiation of some myeloid leukemias [27], growth and migration of endothelial cells [7], increase in osteoclastic activity and decrease in osteoblast activity [28].

G-CSF alters sympathetic tone directly and decreases norepinephrine reuptake, leading to longer duration signals transmitted by the sympathetic nervous system [29]. The adrenergic signals play a key role in the G-CSF release of HSC [30].

Clinical usefulness of G-CSF/G-CSFR

Oncological importance and utilization of G-CSF/ G-CSFR

In recent years there has been a huge progress in understanding the role of certain biomolecules during the synthesis and activation of blood cells; now the research is focusing more and more on the proteomics and genomics domains. Polymorphonuclear neutrophils (PMN) play an important role in the innate immune system, being the most numerous of the system an acting as the first guarding against infections. Recent investigations have highlighted the potential antitumor efficacy of (PMNs). Restoring the function of PMN (or enhancing it) following the administration of G-CSF or GM-CSF in cancer patients could represent a new therapy direction. Some cancer types exhibit an oncogene profile that makes them sensible to PMN actions [31]. Although tested in small-scale clinical trials, the efficiency of GM-CSF as a single agent showed low frequency complete remission [13].

Being among the first cytokines identified, G-CSF was rapidly introduced into clinical medicine. It was initially used to minimize chemotherapy-induced myelosuppression due to its effect on the production and maturation of neutrophils [19]. G-CSF and GM-CSF are effective at reducing the neutropenia duration and the risk of neutropenia-related negative events [32], reducing the risk of febrile neutropenia and early deaths, including infection-related mortality [33]. The association of CSF with antibiotics does not influence the overall mortality in patients with chemotherapy-induced febrile neutropenia. Benefits of this therapy are the reduced time of hospitalisation and the faster recovery of the immune system due to the neutrophils recovery [34]. Primary prophylaxis with a G-CSF is more common in breast cancer, lung cancer and non-Hodgkin's lymphoma [35].

G-CSF is able to mobilize hematopoietic stem cells from the bone marrow into the blood, therefore it can be used in hematological malignancies for hematopoietic stem cell transplantation, before application of myeloablative therapy or for increasing chemosensitivity [19].

The oncological importance of G-CSFR is proved by several facts: 20% of cases of severe congenital neutropenia present mutations of the gene encoding the G-CSFR [36], approximately 80% of patients with congenital neutropenia who have developed acute myeloid leukemia possess mutations in the G-CSFR [37]; the type B acute leukemia cells expresses a small number of G-CSFR, and are not responsive to the action of G-CSF [38].

Unfortunately, therapy with G-CSF involves some adverse events: in acute leukaemias it can increase drug resistance to daunorubicin [39]. In a study of Hershman et al. the risk for developing myelodysplastic syndrome or acute myeloid leukemia for the patients with breast cancer treated with G-CSF was higher than for the ones not receiving it [40]. Another rare but serious adverse event of the therapy with G-CSF is the spleen rupture [41].

Other therapeutic indications for G-CSF

The therapeutic indications of G-CSF are continuously increasing:

- non neutropenic patients infections: pneumonia, diabetic foot infection, wound, fungal infections [1];
- reproductive medicine: human recombinant G-CSF as innovative therapy for infertile patients, folicular G-CSF as biomarker of competence and quality of oocyte, and human recombinant GM-CSF for supplementing the embryo culture [2];
- neurological disturbances (e.g., cerebral ischemia, stroke, neuronal injury, and more recently PD), due to the activating effect of some neuroprotective pathways, such as mobilization of neuronal differentiated hematopoietical stem cells, initiation of angiogenesis, anti-inflammatory and antiapoptotic effects. But this novel trophic recovery treatments is conditioned by the early intervention during such disturbances [3];
- therapy of acute myocardial infarction due to the anti-apoptotic effect on damaged myocardium [42];
- regeneration therapy for skeletal muscle by stimulating myoblast proliferation [43] and hepatitis C therapy, for neutropenia associated with hepatitis C virus therapy [44].

Some engineered chimeric cytokines and myelopoietins which have a unique biologic effect and potency are well used in therapy. These contain agonists for interleukin-3 receptors and granulocyte colony-stimulating factor receptors [45].

Conclusions

The main usefulness of G-CSF therapy is represented by the primary prophylaxis of febrile neutropenia and infections induced by oncological chemotherapy.

The regeneration therapy for neurons, myocardium, and skeletal muscle, the reproductive medicine and hepatitis C therapy represent new directions for G-CSF therapeutic indications.

New engineered cytokines drugs containing G-CSF

have stronger potency and are promising for clinical trials in cytokine's clinical medicine.

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