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New insight into the mechanism of granulocyte colony-stimulating factor (G-CSF) that induces the mobilization of neutrophils

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ABSTRACT

Over the past 20 years, granulocyte colony-stimulating factor (G-CSF) has driven the attention of researchers as a therapeutic agent for curing patients suffering from neutropenia. Despite the successful use of G-CSF, it currently requires daily injections, which are inconvenient, expensive, and distressing for children. Therefore, an alternative strategy for using G-CSF for treatment is needed. Understanding the G-CSF structure, expression, mechanism of action, and how it induces neutrophils mobilization is crucial to producing promising cancer therapy. The ability of G-CSF to mobilize hematopoietic stem cells from the bone marrow into the blood circulation was consequently exploited and altered the practice of hematopoietic stem cell transplantation. This is the motivation for the current review, which sheds light on the history of G-CSF and then focuses on the mechanism of action upon binding to its receptor (G-CSFR) and how that had led to the stimulation of neutrophils mobilization. The findings of this review show new insight into the mechanism of G-CSF that induces neutrophils mobilization. Thus, Understanding the G-CSF will provide a more effective treatment for all neutropenia patients.

KEYWORDS

G-CSF; neutrophils

1. History of granulocyte colony-stimulating factor (G-CSF)

In the early 1960s, several studies on animal models were conducted to explore how white blood cells (WBCs) are regulated within the blood circulation. In 1966, the identification of WBCs specific regulator remained unknown until two research groups developed an *in-vitro* assay that measured the growing colonies of granulocytes and monocytes from bone marrow (BM) and spleen cells samples [1,2]. Nevertheless, the growth of colonies was based on the presence of unknown proteins that were given the name of colony-stimulating factors (CSFs). In the middle of the 1980s, different laboratories performed work to purify and classify CSF proteins. Resulting from these efforts, four CSF proteins with different activities were discovered. They were classified and named based on the type of cell colonies they stimulated: granulocyte-macrophage colony stimulating factor (GM-CSF) stimulated both granulocyte and macrophage colonies, macrophage colony stimulating factor (M-CSF) stimulated macrophage colonies, G-CSF stimulated granulocyte colony formation, and multi-CSF (known as interleukin 3, IL-3) stimulated multiple hematopoietic cell colonies [3].

In the middle of 1983, G-CSF was first purified and characterized in mice using a mouse lung-conditioned medium by Nicola and his collaborators in Melbourne, Australia [4]. After two years, Human GCSF (hG-CSF) was first purified from the human bladder carcinoma cell line 5637 [5]. In 1986, molecular cloning of the complementary deoxyribonucleic acid (cDNA) for G-CSF and the first expression of Escherichia coli (*E. coli*) were attained by Souza and Boone [6]. As a result, the previous accomplishments facilitated the development of recombinant G-CSF, which enabled the study of its biological characteristics [7].

2. G-CSF

2.1. Structure of G-CSF

Human G-CSF (hG-CSF) is located on chromosome 17 and encoded by CSF3 gene. However, this gene encodes two different messenger ribonucleic acid (mRNA) products due to G-CSF differential splicing: G-CSFa contains 177 amino acids (18.8kD) and G-CSFb contains 174 amino

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acids (19.6kD). The difference between the two types is that G-CSFa contains additional three residues after Leucine35 (Valine-Serine-Glycine). The G-CSFb (174 amino acids) contains a glycosylation site on the oxygen known as O- linked glycosylation. It is attached to one threonine at the site of residue 133, and this form is expressed in mammalian cells. It has been reported that G-CSFb obtains more biological activity (~20 times more than G-CSFa), which makes it the source of commercial pharmaceutical products for G-CSF [8].

The feature of hG-CSF central structure is similar to other helical cytokine family members. It contains four antiparallel, left-handed α-helical fold bundles connected by two long loops in a form that two helices extend up (A contains 29 amino acids & B contains 21 amino acids) and two helices extend down (C contains 24 amino acids & D contains 30 amino acids) [9] (Figure 1). Additionally, hG-CSF has five cysteine residues. Four of these cysteines form two internal disulfide bonds located between Cys36– Cys42 and Cys64– Cys74, leaving one free cysteine residue at Cys17th position with a free sulfhydryl group [10].

The central structure of hG-CSF consists of four antiparallel, left-handed α-helical fold bundles connected by two long loops in a form called up–up–down-down structure that two helices A (Red color) and B (Orange color) extend up and two helices C (White color) and D (Cyne color) extending down. N = amine-terminus and C = carboxyl-terminus (Molecular graphics system called PyMOL) (Figure 1).

2.2. The importance of G-CSF expression and action

hG-CSF is a cytokine that regulates the proliferation, differentiation, and survival of neutrophils [11], as proved by the significant reduction of neutrophils in both G-CSF and G-CSFR deficient mice [12,13]. G-CSF can be produced by a variety of cells, including endothelial cells, fibroblasts, macrophages, monocytes, and bone marrow stromal cells in response to several inflammatory mediators such as vascular endothelial growth factor (VEGF), interleukin β (IL-1β), interleukin (IL-17), lipopolysaccharide (LPS), necrosis factor alpha (TNF-α) [14]. It has been reported that hG-CSF is highly expressed on a number of cancer cell types, including human gastric and colon cancers [15,16], as well as acute myeloid leukemia (AML) [17,18] and other different carcinoma cells [19]. Besides, it has a major role in treating neutropenia in cancer patients undergoing chemotherapy [20].

G-CSF is present at low levels in healthy individuals and at increased levels in infections and inflammations, etc [21–23]. Normally, levels of circulating G-CSF are very low (<100 pg/mL). Nevertheless, G-CSF levels can increase to 20 times baseline levels in conditions of stress, resulting in a rapid increase of circulating neutrophils [24].

Many mechanisms or evidences revealed that G-CSF could stimulate and regulate the production of neutrophils progenitors from the bone marrow to the blood circulation. G-CSF enhances the proliferation of all granulocytic lineages from myeloblast (hematopoietic stem cell) to myelocyte. GCSF drives differentiation of neutrophils and rapidly accelerates the metamyelocytes maturation, resulting in rapid and continuous elevation of neutrophils number in the blood circulation [25–28]

Significantly, it has been shown that G-CSF treatments stimulate a faster erythropoiesis-enhancing response than that of erythropoietin (EPO). These data recommend an alternative method to treat acute anemia, especially when patients undergo a clinical emergency in remote areas without appropriate supplies from blood banks [29].

Recently, it has been reported that G-CSF has a dual activity that is beneficial both in decreasing acute neuronal degeneration and enhancing long-term plasticity following cerebral ischemia in the CNS. Treatment of G-CSF exerts neuroprotective effects on damaged neurons throughout the suppression of the mitochondrial stress and endoplasmic reticulum (ER) stress and maintains cellular homeostasis by reducing pro-apoptotic proteins and increasing anti-apoptotic proteins [30].

3. G-CSF receptor

3.1 Discovery, expression, and cloning of G-CSF receptor

Effects of G-CSF are mediated by binding to a single homodimer receptor, granulocyte colony stimulating factor receptor (G-CSFR). Therefore, the regulation, proliferation, and differentiation of neutrophils precursors are highly dependent upon binding to their receptors [15]. GCSF-R is a membrane protein expressed in all granulocytic lineage cells, including neutrophils, progenitors, and myeloid leukemia cells [31]. G-CSFRs have also been detected on normal B & T lymphocytes, monocytes [32,33], and non-hematopoietic tissues, such as cardiomyocytes [34], vascular endothelial cells [35], neural stem cells [36], placenta [37], and many non-hematopoietic tumors cell lines [38].G-CSFRs are mainly expressed on common myeloid precursors (CMP), and mature neutrophils, however, the expression of these receptors increases more during maturation [39]. Recently, G-CSFRs have been shown to be highly expressed on human gastric and colon cancer cells [16].

In 1990, G-CSFR was first cloned from mice myeloid leukemia cell line (NFS-60) and shown to form homodimers upon binding to its ligand G-CSF, resulting in a complex 2:2 ligands: receptor subunit [40].

3.2. G-CSFR structure and function

The G-CSFR is a cell surface receptor and belongs to the class I hematopoietic cytokine receptor super-

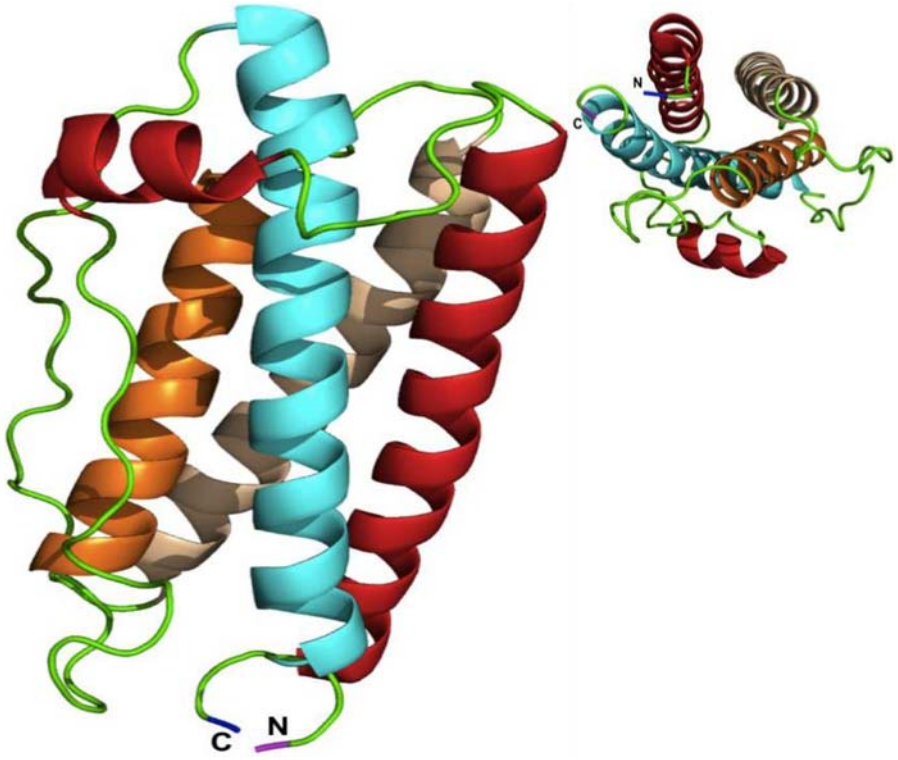

Figure 1. Human G-CSF structure.

family (HCR) [41]. G-CSFR is around 120 kDa and contains 813 amino acids in length, arranged as follows: an extracellular region (604 amino acids), a transmembrane region (26 amino acids), and 183 amino acids for an intracellular cytoplasmic domain. The extracellular domain consists of N-terminal immunoglobulin (Ig)-like domain, cytokine receptor homology (CRH) domain, and a Trp-Ser-X-Trp-Ser (WSXWS) motif required for G-CSF ligand binding (a hallmark of the class I cytokine receptors), and the remainder of this region is formed by 3 fibronectin type III (FNIII) domains [42–44].

The intracellular region contains three conserved sub-domains called Box 1, Box 2 and Box 3 [45]. Box 1, Box 2, and tyrosine residues at site Y704 have a fundamental role in proliferating the signaling, while the Box 3 motif is associated with receptor trafficking [46,47]. The intracellular region also has three tyrosine residues, 729, 744, and 764, which play an important role in proliferation, differentiation, and cell survival (Figure 2) [43,46].

Ligation of G-CSF to its receptor forms homo-dimers with stoichiometry 2:2 [48,49]. Each ligand of G-CSF interacts with the CRH domain of one G-CSFR subunit and the immunoglobulin (Ig)-like domain of the second G-CSFR subunit, forming a crossover configuration of the receptor subunits (Figure 3(B)) [50].

3.2.1. JAK/STAT pathway

The binding of G-CSF causes conformational changes to its receptor that activates the Janus kinase (JAKs)/signal transducer family JAK1, JAK2, and Non-receptor tyrosine-protein kinase (TYK2) [46]. The activation of JAKs proteins subsequently phosphorylates G-CSFR by binding to its Box 1 and 2 domains, creating potential docking sites for a variety of signaling molecules such as signal transducer and activator of transcription (STAT) proteins in cytoplasm [51], particularly STAT3 with slight stimulation of STAT1 and STAT5 [43,52]. G-CSFR dimerizes and brings the JAKs together into proximity resulting in their trans-phosphorylation of one another. This, in turn, phosphorylates tyrosine (Y) residues (Y704, Y729, Y744, and Y764) located in the cytoplasmic region and serves as docking sites for STAT's. Notably, STAT3s have been reported to interact with tyrosine residues 704 and 744 of the G-CSFR through their Src Homology 2 (SH2) domains, get phosphorylated and activated by JAK2, and then form homodimers that migrate to the nucleus, where they bind DNA and activate gene transcription [53–58]. It seems that they might regulate the mobilization of neutrophils from the bone marrow to the blood circulation [59,60]. In normal conditions, STAT3 induces a suppressor of cytokine signaling 3 (SOCS3) to bind and activate G-CSFR, leading to receptor degradation and cessation of the signalling [58,61]. SOCS proteins, mainly SOCS3, showed an inhibitory effect on G-CSF signaling during neutrophilic differentiation [59–61].

STAT5 is another important signalling activated and mediated by G-CSFR that induces proliferation and survival of neutrophils [62], directly controlled and activated by JAKs throughout phosphorylation [61]. However, activation kinetics for STAT5 is significantly

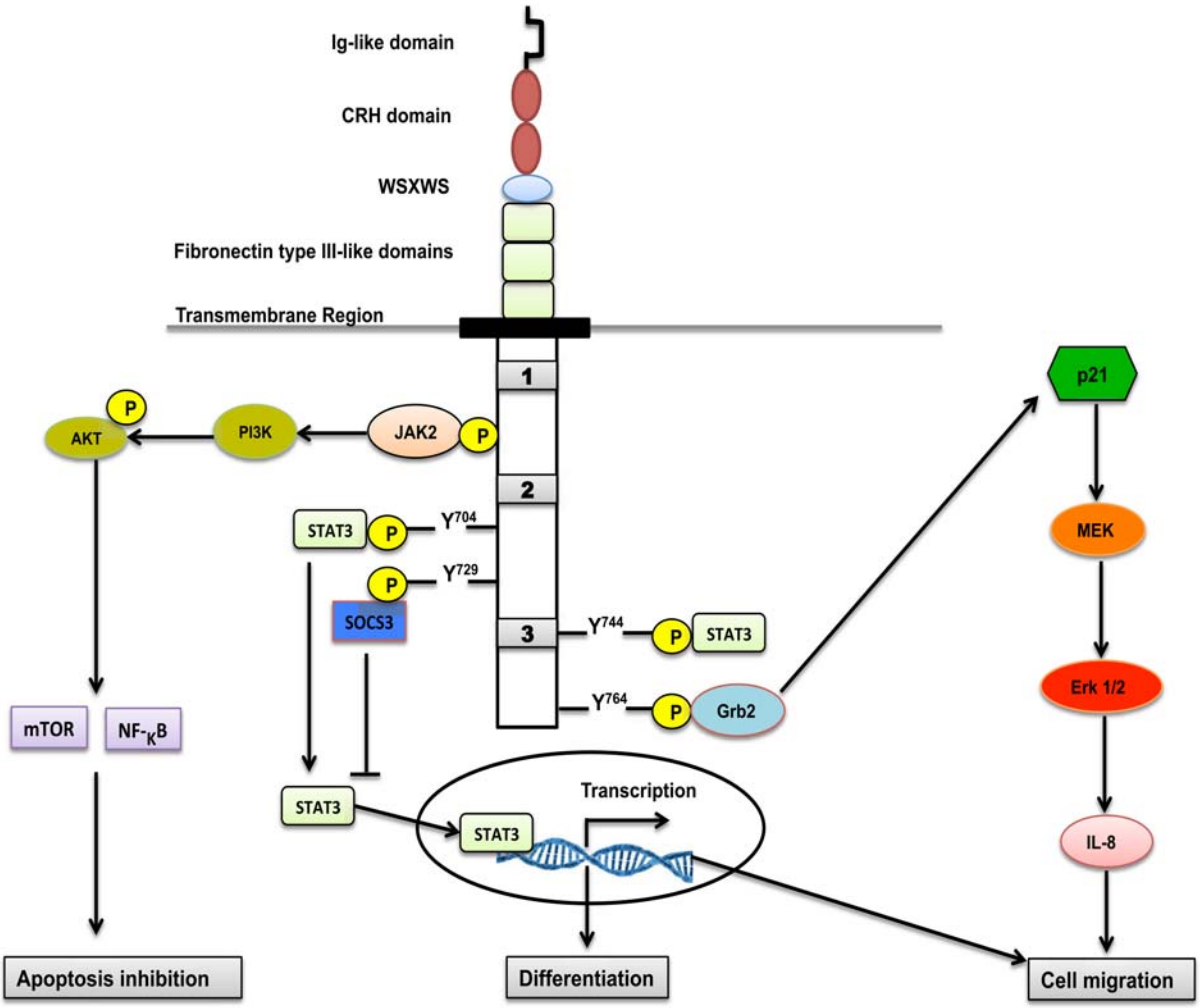

Figure 2. The scheme proposed for domains and downstream signal pathways of G-CSFR.

different from STAT3. The activation of STAT5 is transient and returns to the basal level in around half-hour, while the activation of STAT3 continues for many hours [42,61]. Therefore, binding of G-CSF to its receptor-mediated differential activation of both STAT5 and STAT3 may play an important role in controlling myeloid lineage proliferation against differentiation, especially neutrophils [58]. Since activation of STAT3 is sustained for many hours, it could be the main regulator for the neutrophil's mobilization.

3.2.2. MAPK/ERK pathway

Although ligation of G-CSF to G-CSFR is widely believed to induce JAK/STAT pathways, it has also been linked to mitogen-activated protein kinases/ extracellular signal-regulated protein kinase MAPK/ ERK pathway (also recognized as the Ras-Raf-MEK-ERK pathway). Trosine residue Y764 serves as a docking site for the growth factor receptor-bound protein 2(Grb2), which induces p21 Ras pathway. *In vitro,* a significant decrease in the activation of p21 Ras and proliferation of neutrophils were noticed when Y764 was absent [63,64]. (Erk 1/2) MAP kinase is considered to be the main downstream effector from the p21 Ras pathway that is involved in signaling proliferation of myeloid precursor cells. It is also reported that Erk1/2 is strongly activated in neural cells upon exposure to G-CSF [7,42,65]. The binding of G-CSF to its receptor activates the intracellular kinase (MEK). Phosphorylated MEK stimulates Erk ½ to be activated. Phosphorylated Erk ½ induces neutrophils migration and IL-8 production [55].

3.2.3. AKT/PI3-K

AKT/ PI3-K signalling is another pathway that is activated by JAK2. It is important for differentiation, proliferation, and survival of immature neutrophil precursors by activating the nuclear factor kappa B/ mammalian target of rapamycin (NF-κB, mTOR), which acts as an inhibitor for apoptosis but is not able to extend the lifespan of neutrophils in the presence of G-CSF (Figure 2) [66].

Mobilization of neutrophils

Production, proliferation, and differentiation of neutrophils initiate from the HSCs in the bone marrow. It has been shown that following differentiation; the majority of mature neutrophils remain in the bone marrow for

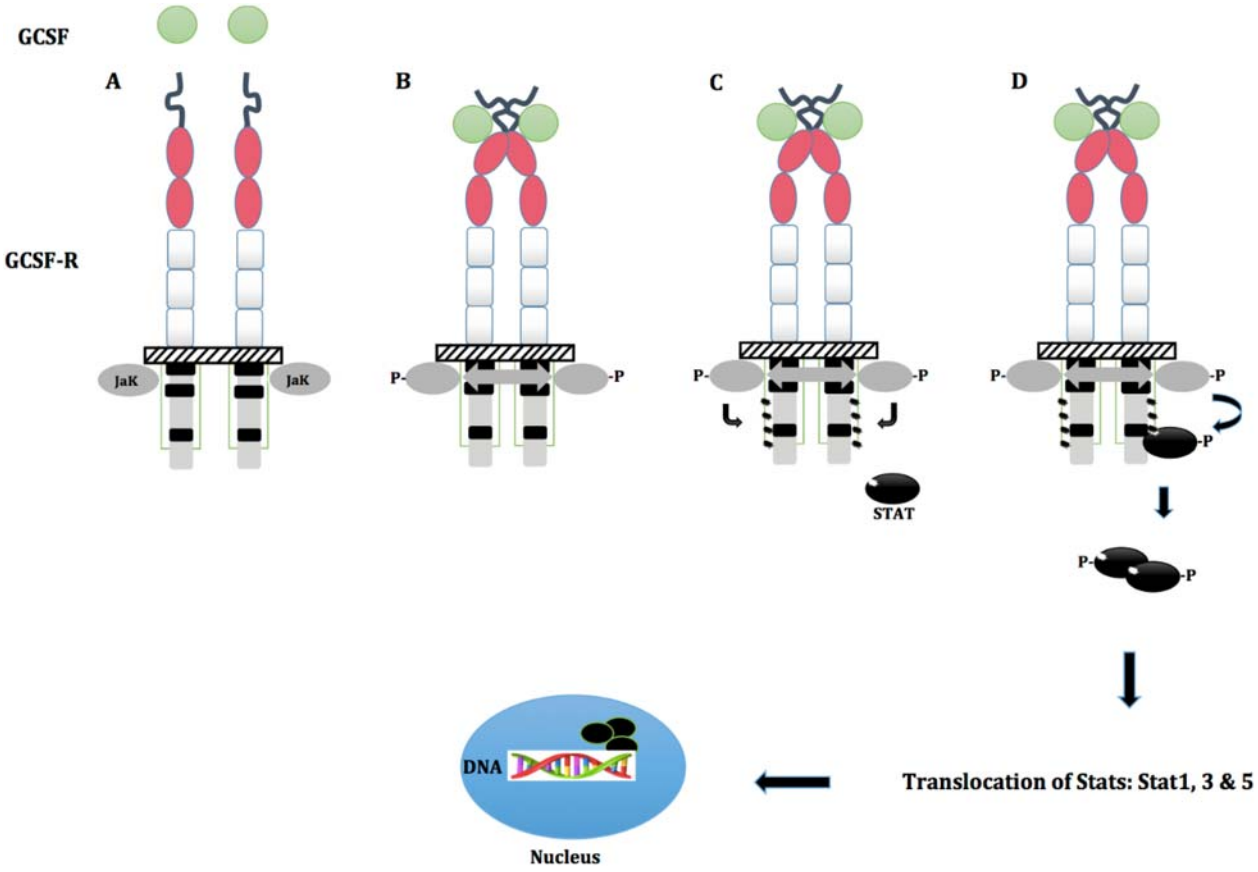

Figure 3. [A] In the absence of the ligand, G-CSFR is associated with Janus kinases (Jaks). [B] The binding of the ligand to the receptor occurs at a 2:2 ligand:receptor subunit stoichiometry, forming a crossover configuration between the receptor subunits that brings the Jaks into proximity and enables their trans-phosphorylation and stimulation. [C] The intracellular 4-tyrosine residues of the G-CSFR (represented by stars) are phosphorylated by Jaks. [D] STAT interacts with the phosphotyrosine residues through their Src Homology 2 (SH2) domains and become phosphorylated by the Jak. Phospho-dimers of STATs accumulate in the nucleus and activate transcription factors that drive the neutrophils from the bone marrow to the blood circulation.

approximately 5–6 days. During infection or inflammation, these cells are available for rapid response and release to the blood circulation and then migrate to affected tissues [67].

G-CSF is a hematopoietic cytokine that regulates granulopoiesis and promotes proliferation, differentiation, and neutrophil activation [14]. It has been shown that G-CSF enhances neutrophils' migration into the peripheral tissues. In addition to G-CSF, different agents are involved in the regulation of neutrophils migration. For example, C5a, leukotriene B4 (LTB4), and CXCR2 ligands (e.g. IL-8) are found in humans, whereas keratinocyte chemoattractant [KC] and macrophage inflammatory protein 2 [MIP-2] in mice; [68–70]. It has been shown that CXCR2 is more potent than G-CSF in enhancing neutrophils migration [71].

Previously, it was reported that STAT3 is the major transcription factor activated upon binding of G-CSF to its receptor, but the role of STAT3 in the mechanism of neutrophils mobilization was not clear [72]. At the early stage of acute inflammation, the chemokines, macrophage inflammatory protein-2 (MIP-2, known as Cxcl2) and keratinocyte-derived chemokine (KC, Cxcl1), and their shared receptor CXCR2 induce the mobilization of neutrophils from the BM to the circulating blood. A previous study examined the role of STAT3 for neutrophil migration by treating STAT3-deficient mice with MIP-2 by intraperitoneal injection. Treated STAT3-deficient mice did not show an increase in circulating neutrophil amounts compared to wild-type mice, which suggested the importance of STAT3 in neutrophils migration [59,60,73].

Bajrami et al. [67,74] showed that G-CSF does not synergize with CXCR2 to induce neutrophil mobilization during the early phase of acute inflammation. Instead, it inhibits CXCR2-mediated rapid neutrophil mobilization. This result verifies that the initial CXCR2-mediated neutrophil mobilization can occur at maximal levels without G-CSF–induced inhibition. At a later-phase of acute inflammation, G-CSF could induce STAT3 to suppress CXCR2-mediated rapid neutrophil mobilization and reserved neutrophils in the BM, in part throughout their chemokine receptor 4 (CXCR4) expression, which binds to the stromal cell-derived-1 (SDF-1), expressed in the BM [59,60,73] (Figure 4).

In summary, because G-CSF itself is not chemotactic, this concept is supported by the observation that

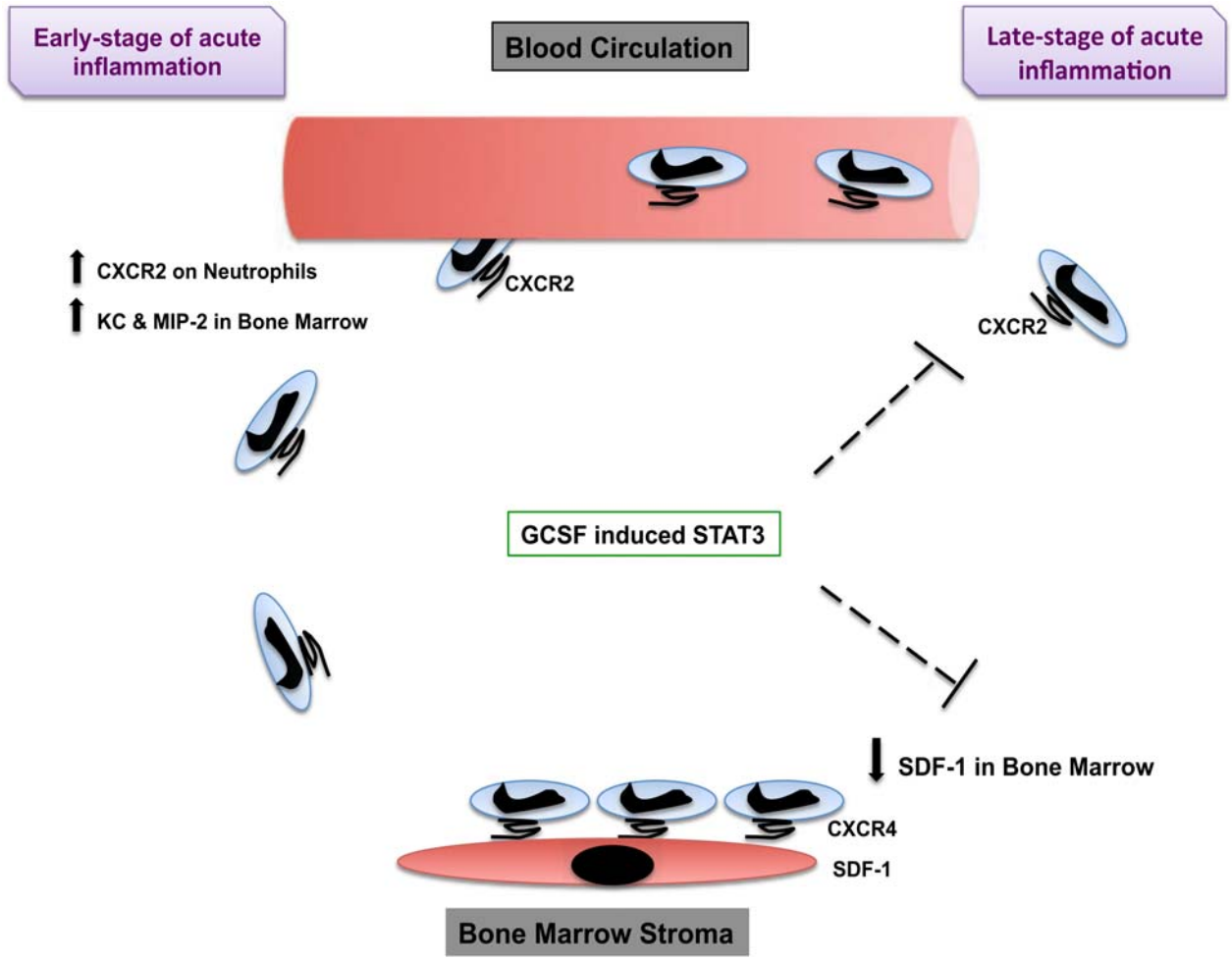

Figure 4. Scheme of the proposed model. How G-CSF and STAT3 induce the mobilization of neutrophils. At the early stage of acute inflammation, MIP-2, KC, and their shared receptor CXCR2 induce the mobilization of neutrophils from the BM to the blood circulation. At the late stage of acute inflammation, GCSF, together with STAT3, inhibits CXCR2-mediated rapid neutrophil mobilization and reserved neutrophils in the BM, in part throughout their CXCR4 expression, which binds to SDF-1 (stromal cells express SDF-1) expressed in the BM. (↓ reserve ؛ ↑ induce).

G-CSF fails to induce circulating neutrophil amounts in CXCR2-knockout mice [74]. Inhibiting the SDF-1/CXCR4 interaction is sufficient to enable neutrophil release from the marginated pool present in the lung and block the neutrophil trafficking back to the BM, as shown by the use of the CXCR4 antagonist AMD3100 (Plerixafor) [75].

Conclusions

G-CSF is a hormone produced by different tissues to stimulate neutrophils' production from the bone marrow into the blood circulation. The rhG-CSF has been shown to stimulate neutrophils to treat neutropenic patients and in stem cell mobilization in the cases of BM transplantation. Nowadays, G-CSF is a recognized therapy routinely used to treat patients with neutropenia and thus decreasing morbidity, especially cancer patients undergoing chemotherapeutic drug treatments. Understanding the G-CSF structure, expression, and mechanism of action upon binding to its receptor and leading to the mobilization of neutrophils is fundamental to generating a promising cancer therapy.

Declaration

Ethics approval and consent to participate

This study doesn't contain any human materials so there is no need for consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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