HHS Public Access

Author manuscript

Curr Opin Hematol. Author manuscript; available in PMC 2015 June 03.

Published in final edited form as:

Curr Opin Hematol. 2009 January ; 16(1): 35–40. doi:10.1097/MOH.0b013e328319913c.

Effects and safety of granulocyte colony-stimulating factor in healthy volunteers

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Abstract

Purpose of Review—Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is now widely used in normal donors for collection of peripheral blood progenitor cells (PBPCs) for allogeneic transplantation and granulocytes for transfusion. Currently available data on biologic and molecular effects, and safety of rhG-CSF in normal healthy volunteers are reviewed.

Recent Findings—In addition to its known activating role on neutrophil kinetics and functional status, rhG-CSF administration can affect monocytes, lymphocytes and the hemostatic system. G-CSF receptors were identified in a variety of non-myeloid tissues, although their role and functional activity have not always been well defined. Moreover, rhG-CSF is capable of modulating complex cytokine networks and can impact the inflammatory response. In addition to its known mobilizing role for PBPCs, rhG-CSF can mobilize dendritic and endothelial progenitor cells as well. On a clinical level, serious rhG-CSF-related adverse events are well described (e.g. splenic rupture) but remain rare.

Summary—rhG-CSF effects in healthy volunteers, while normally transient and self-limiting, are now believed to be more complex and heterogeneous that previously thought. While rhG-CSF administration to healthy volunteers continues to have a favorable risk-benefit profile, these new findings have implications for safeguarding the safety of normal individuals.

Keywords

Normal donors; granulocyte colony-stimulating factor; G-CSF; filgrastim

INTRODUCTION

The administration of recombinant human granulocyte colony-stimulating factor (rhG-CSF) to healthy volunteers is now commonly carried out for peripheral blood progenitor cell (PBPC) mobilization [1,2, 3*]. In addition, rhG-CSF is routinely administered to volunteer donors to collect granulocytes [4*]. The effects and safety of rhG-CSF in healthy volunteers have been discussed [1]. This review will concisely summarize newly available data on

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Funding disclosure: No relevant funding source to disclose.

biologic and molecular effects, as well as safety of rhG-CSF in normal healthy volunteers. It will also outline some of the new and unique set of challenges they create.

THE G-CSF RECEPTOR (G-CSFR)

The G-CSFR is a type I membrane protein belonging to the cytokine receptor superfamily. It is a single-chain polypeptide and is encoded by a single gene on chromosome 1p35-p34.3 [5,6,7**,8]. The G-CSFR is present on pluripotent and myeloid-committed progenitors, as well as differentiated myeloid cells from the myeloblast to the mature neutrophil [5, 6, 7**, 8]. The number of G-CSFRs increases with maturation within the myeloid lineage and neutrophils have the greatest number. However, even neutrophils express a relatively low number of G-CSFRs (about 50-500 per cell), and it is believed that only a minority of these receptors needs to be occupied to elicit a maximum biologic response [5, 6].

The presence of G-CSFRs is not restricted to myeloid cells as originally thought, although the biologic effects of G-CSFR expression in non-myeloid tissues remain uncertain. Several investigators have reported on the presence of G-CSFRs on subsets of monocytes and lymphoid cells, platelets, vascular endothelial cells, human placenta, trophoblastic cells and possibly neurons, glial cells and hepatocytes as well [9-12,13**,14**]. The role and functional significance of these receptors in non-myeloid tissues require further study.

HEMATOLOGIC EFFECTS OF rhG-CSF

Neutrophil kinetics and functional status

RhG-CSF-induced effects on neutrophils are well described [5, 15-17]. RhG-CSF stimulates the proliferation of myeloid precursors, accelerates neutrophil release from the bone marrow. It mobilizes secretory vesicles, and induces the release of the contents of specific and azurophilic granules. RhG-CSF activates neutrophils enhancing their phagocytic function, including respiratory burst metabolism, surface CD11b/CD18 antigen expression and cellular elastase activity [5, 15-17].

Monocytes

A five-day course of rhG-CSF will lead, on average, to a three-fold increase in the number of peripheral blood (PB) monocytes in PBPC donors. This is associated with monocyte activation and modulation of effector molecules on monocytes [18]. There is evidence for the presence of G-CSF receptors on at least some monocyte subsets [9,18]. Monocytes from rhG-CSF-treated normal subjects produce more IL-10 than unmobilized monocytes in response to pro-inflammatory factors such as lipopolysaccharide (LPS) [19]. Fraser et al [20] studied monocyte phenotype, as well as IL-10 localization and release in rhG-CSF-treated healthy donors. IL-10 preferentially bound to the surface of a subset of immature monocytes (CD64/CD14$^{low/neg}$), and the ability of these cells to stimulate alloreactivity was blunted.

Eosinophils

rhG-CSF administration to healthy individuals raises the number of circulating eosinophils, affects the mobilization of eosinophil granule proteins and enhances eosinophil adhesion [15, 21].

Platelets and coagulation

Data on the effects of rhG-CSF on platelet function in normal subjects are limited, and it should be acknowledged that platelet function and aggregation studies are frequently difficult to interpret due to the paucity of standardized and uniformly accepted testing methods. Shimoda et al [11] identified functional G-CSF receptors on platelets. They also found increased ADP-induced platelet aggregation in four rhG-CSF-treated normal volunteers [22]. Another study found reduced platelet aggregation [23].

The impact of rhG-CSF treatment on the coagulation system in normal donors has been evaluated in more detail. Falanga et al [24] reported an impact of rhG-CSF on hemostasis in normal donors. They showed an increase in plasma markers of endothelial activation (thrombomodulin and von Willebrand factor antigens) and blood coagulation activation (F1+2, TAT complex, D-dimer), as well as endotoxin-induced mononuclear cell procoagulant activity. These changes were largely resolved one week after stopping treatment. Topcuoglu et al [25] reported similar findings. Leblanc et al [23] found increased levels of Factor VIII:C and thrombin generation in normal donors after rhG-CSF administration. Söhngen et al [26] detected increased Factor VIII and fibrinogen levels, while protein C and protein S activities were reduced. These data suggest that rhG-CSF may induce a transient prothrombotic or hypercoagulable state in some normal donors. Whether healthy donor screening for thrombophilic conditions and prophylaxis with low-molecular weight heparin should be undertaken remains unclear [27*]. Rare donors with vascular events have been reported during or shortly after PBPC donation [28*].

IMMUNOLOGIC EFFECTS OF rhG-CSF

Lymphocytes

The presence of G-CSFRs on lymphocytes has been controversial. Franzke et al [10] detected G-CSFR expression in Class I and Class II-restricted T cells both in vivo and in vitro in normal donors. While G-CSFR gene expression in T cells was undetectable before rhG-CSF stimulation, it could be repetitively induced for up to 72 hours after rhG-CSF stimulation. The gene expression profile in T-cells was modulated by rhG-CSF exposure. There was upregulation of surface activation markers (CD69 and CD53) and transcription factors (GATA-3), accompanied by downregulation of costimulatory (CD5 and CD44) and adhesion (LFA-1α) molecules. Boneberg et al [9] were unable to identify G-CSFRs on lymphocytes.

Much has been written on rhG-CSF ability to “polarize” T-cell subsets and reduce alloreactivity, while inducing T-cell tolerance in normal donors [29,30]. Lindemann et al [31] showed that rhG-CSF administration to normal donors suppresses cellular immune function within days and increases soluble HLA antigen levels. These effects resolved by

two months of treatment. In another study, donor treatment with pegylated G-CSF augmented the generation of IL-10-producing regulatory T-cells [32]. RhG-CSF induced a humoral-mediated perturbation of mitochondrial function and DNA in lymphocytes of rhG-CSF-treated donors, leading to inhibition of cell cycle progression [33].

Natural Killer (NK) cells

One study [34] showed no effect of rhG-CSF administration on the PB NK cell phenotype (defined as $CD3^-/CD56/CD16^+$ or $CD3^-/CD56^+$ cells) of adult PBPC donors.

EFFECTS ON CYTOKINE RESPONSES AND NETWORKS

Many of the rhG-CSF-induced biologic and molecular effects in normal donors outside the myeloid lineage may be accounted for by the transient modulation of cytokine responses and networks. Weiss et al [35] reviewed in detail the impact of exogenous G-CSF on the main cytokine pathways of the systemic inflammatory response. RhG-CSF administration may have both pro-inflammatory and anti-inflammatory effects [35].

Boneberg et al [9] identified functional G-CSFRs on PB monocytes of rhG-CSF-treated healthy subjects. They found a decrease in the release of the proinflammatory cytokines tumor necrosis factor (TNF-α), interleukin(IL)-12, IL-1β, interferon (IFN)-γ from lymphocytes in ex-vivo LPS-stimulated whole blood. rhG-CSF had no effect on IFN-γ release from isolated lymphocytes. The authors concluded that the attenuation of IFN-γ release from lymphocytes was not due to a direct effect of rhG-CSF on these cells, but rather to the inhibition of IL-12 and TNF-α release from monocytes by rhG-CSF. Franzke et al [10] showed that T-cell exposure to rhG-CSF is associated with spontaneous IL-4 release. Lindemann et al [31] have shown a significant rise in IL-10 plasma levels in volunteer PBPC donors. A surge in IL-8 serum levels following rhG-CSF administration was described by Watanabe et al [36] [36].

EFFECTS ON CELL MOBILIZATION

PBPCs

RhG-CSF ability to mobilize PBPCs in normal donors was evaluated in detail and the mechanism(s) involved recently reviewed [37*]. The homing of hematopoietic progenitors to the marrow microenvironment involves adhesion molecules. Mobilization is mediated, at least in part, by metalloproteases released from myeloid cells upon rhG-CSF stimulation [37*]. Saito et al showed that rhG-CSF administration to healthy volunteers results in elevated serum levels human matrix metalloproteinase-9 (MMP-9) [38*]. Tagami et al reported similar findings for high mobility group box 1 (HMGB1), a non-histone protein involved in cell migration and stem cell recruitment [39]. Adhesion molecule shedding is believed to occur with mobilization, and two studies in normal donors have shown increased serum levels of soluble adhesion molecules (sL-selectin, sE-selectin, sCD44 but not sICAM-1) following rhG-CSF administration [40,41].

Dendritic cells

Another established effect of rhG-CSF in PBPC donors is represented by its ability to mobilize dendritic cells (DCs) [42,43]. Mature DCs are antigen-presenting cells involved in the cellular immune response, and have a unique ability to stimulate naïve T cells. Two separate lineages of DCs have been described in humans (DC1 and DC2), according to their ability to trigger naïve T-cell differentiation to Th1 and Th2 cells, respectively [42]. The monocyte-derived $CD11c^+$ DCs induce T-cells to produce Th1 cytokines in vitro, whereas the plasmacytoid T-cell-derived $CD11c^-$ DC subset stimulates the production of Th2 cytokines [43].

Arpinati et al [42] studied rhG-CSF treated normal donors, as well as normal controls. While the rhG-CSF-treated donors were not employed as their own controls and a different set of control samples was used, they found a 5-fold increase in the PB DC2 counts, while the DC1 count was unchanged. Pulendran et al [43] studied rhG-CSF-treated healthy volunteers, employed as their own controls. They found that rhG-CSF only increased (> 7-fold) the plasmacytoid T-cell-derived $CD11c^-$ DC subset. Proinflammatory slanDCs (6-sulfo $LacNAc^+$ DCs) represent an important source of IL-12 and TNF-α. They are mobilized by rhG-CSF in healthy volunteers, and retain their ability to release IL-12 and TNF-α [44*].

Endothelial progenitor cells (EPCs)

rhG-CSF also mobilizes EPCs, which may have significant implications for tissue regeneration and angiogenesis [45,46*]. EPCs coexpress surface CD34, CD133 and vascular endothelial growth factor receptor 2 (VEGFR-2) antigens in vivo. In a study of healthy rhG-CSF –treated donors, the median PB blood concentration of CD34+133+ VEGFR-2-positive EPCs increased eight-fold from steady state, and the concentration of CD34+, CD133-VEGFR-2-positive EPCS increased ten-fold [45]. This study is provocative, but it is a small one (eight donors) and cell subsets were defined solely based on surface markers, not functional characteristics. Allan et al [46*] looked at vascular progenitor cells (VPCs) mobilization in normal donors (n=21) as well as in patients receiving cyclophosphamide plus G-CSF. They employed functional assays, and found that VPC mobilization occurred independently of hematopoietic progenitor mobilization. Interestingly, in a study involving CAD patients, rhG-CSF-induced EPC mobilization was found to be reduced when compared to normal controls [47].

EFFECTS ON CHROMOSOMAL INTEGRITY

Nagler et al [48] studied PB lymphocytes in rhG-CSF-mobilized donors and patients with hematologic malignancies (primarily acute leukemia) with molecular cytogenetics techniques based on fluorescence in situ hybridization (FISH). They found loss of synchrony in allelic replication timing similar to the one detected in the leukemia patients, as well as aneuploidy (monosomy and multisomy) involving chromosome 17. While the loss of replication synchrony was transient, aneuploidy persisted for as long as nine months after rhG-CSF exposure, and was felt to be related to changes in DNA methylation capacity. Questions have been raised on the study methodology and data analysis employed. They include the appropriateness of the controls, the failure to separate malignant cells in the

lymphocyte samples from the leukemia patients, incomplete information on the reproducibility of the assays employed, some lack of clarity around the total data set from which the presented data were extracted as well as the use of a nonconventional statistical significance level.

Kaplinsky et al [49] reported that tetraploid myeloid cells are present in the PB of rhG-CSF-treated donors. Tetraploidy was detected by probes for sex chromosomes and confirmed by secondary FISH on the same cells with autosomal probes. The tetraploidy was detected in up to 0.6% of differentiated myeloid cells (primarily neutrophils and metamyelocytes), although all observed CD34+ cells were diploid. However, interpretation of these results is made difficult by the small sample size of the study, the possibility of a selection bias, as well as the lack of data on untreated controls.

The National Marrow Donor program (NMDP) has reported its experience on this issue. Among 4015 donors who have passed the first anniversary of their PBPC donation, the NMDP has accumulated 9785 years of follow-up (range 1-9 years, with 897 donors ≥4 years). The incidence of cancer in this group was consistent with the age-adjusted U.S. incidence of cancer in the adult population, with no reports of leukemia or lymphoma [50*]. The Spanish Donor Registry recently reported similar follow-up data, albeit with a smaller sample size [51*]. The robust sample size for these data, as well as the adequate follow-up provide significant reassurance, although do not eliminate the need for continued surveillance.

The United Kingdom Donor Registries have proposed a study to screen rhG-CSF-treated normal donors for long-term genetic damage using interphase FISH and array comparative genomic hybridization analysis. Marrow donors will be used as negative controls and patients with hematological malignancies as positive controls [52]. The NMDP is planning a similar study (Confer D, personal communication). The results of these studies will need to be interpreted rationally, with proper clinical correlation, in order to avoid unnecessary negative consequences for donor recruiting and counseling.

EFFECTS ON GENE EXPRESSION PATTERNS

Several groups [53, 54*,55*,56] have reported on the transient modulation of expression for hundreds of genes in normal subjects receiving rhG-CSF as studied by gene microarray technology. A study on nine healthy donors [53] showed upregulation of multiple genes involved in neutrophil activation, as well as downregulation of most genes involved in the immune response, including T-cell-related genes such as genes coding for T-cell receptors and the HLA complex. The alteration in gene expression patterns normalized within two months. In a similar study [54*], about three hundred genes exhibited changes in the level of expression, with gradual normalization over six months. Fifty-three genes involved in cell growth, proliferation and communication gene ontology categories were upregulated (including the gene encoding for the CD34 antigen), while sixty-nine genes were downregulated (including the gene encoding for the CCAAT/enhancer binding protein ε, a transcription factor). Buzzeo et al performed gene microarray analysis on peripheral blood of rhG-CSF-treated donors. Inflammatory and neutrophil activation pathways were

upregulated. Adaptive immune-related gene expression, such as antigen presentation, co-stimulation, T-cell activation and cytolytic effector responses were downregulated [55*]. Shapira et al [56] looked at the extent of double-stranded DNA relaxation and de novo synthesis of DNA in rhG-CSF-treated healthy donors. While both parameters of DNA destabilization were found to be increased, they returned to baseline levels in 1-2 months.

rhG-CSF-RELATED ADVERSE EVENTS

Short-term adverse events related to rhG-CSF administration in healthy volunteers are well described. They include bone pain, headache, myalgias, fatigue, nausea, insomnia, and redness at the injection site [1, 3*,28*]. These are usually mild-to-moderate and self-limiting. Far less common events include splenic rupture, acute lung injury, vascular events and exacerbation of autoimmune or inflammatory conditions. While rare, they can be serious and life-threatening [28*].

CONCLUSION

rhG-CSF effects in healthy volunteers, while normally transient and self-limiting, are likely more complex and heterogeneous that previously thought. They may go beyond a selective impact on the myeloid series and involve additional cell types and tissues. The effects of rhG-CSF on coagulation, inflammation and immunity require additional studies. There may also be important effects related to tissue regeneration and angiogenesis. The issue of a possible impact on chromosomal integrity was raised on the basis of limited, preliminary data, and will need further evaluation. In general, rhG-CSF administration to healthy volunteers has a favorable risk-benefit profile, although PBPC donor monitoring efforts (particularly in the long-term) remain essential.

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