Biologic and molecular effects of granulocyte colony-stimulating factor in healthy individuals: recent findings and current challenges

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Recombinant human granulocyte colony-stimulating factor (rhG-CSF) is widely used in healthy donors for collection of peripheral blood progenitor cells (PBPCs) for allogeneic transplantation and granulocytes for transfusion. The spectrum of its biologic and molecular activities in healthy individuals is coming into sharper focus, creating a unique set of challenges and clarifying the need to monitor and safeguard donor safety. Accumulating evidence indicates that rhG-CSF effects are not limited to the myeloid cell lineage. This may reflect the presence of functional G-CSF receptors on other cell types and tissues, as well as rhG-CSF–induced modulation of cytokine networks. While most rhG-CSF–induced effects are transient and self-limiting, preliminary, provocative data have suggested the possibility of a more durable effect on the chromosomal integrity of lymphocytes. While these reports have not been validated and have been subject to criticism, they are prompting prospective studies and monitoring efforts to determine whether there is a significant risk of long-term adverse events (eg, hematologic malignancies) in healthy PBPC and granulocyte donors. Based on the totality of information that is currently available, the administration of rhG-CSF to healthy donors for the purpose of PBPC donation continues to have a favorable risk-benefit profile. (Blood. 2008;111:1767-1772)

Introduction

Ten years ago, we reviewed the biologic and molecular effects of recombinant human granulocyte colony-stimulating-factor (rhG-CSF) in healthy individuals.1 The publication coincided with the onset of the allogeneic peripheral blood progenitor cell (PBPC) transplantation era, made possible by the administration of rhG-CSF to healthy donors to mobilize and collect PBPCs.2 The use of rhG-CSF for PBPC mobilization has been adopted as an international standard of care and it is now widespread. More than 15 000 stem cell donors are harvested every year, and rhG-CSF-mobilized PBPCs accounted for 75% of related and 50% of unrelated donor donations in North America alone in 2003.3 In addition, rhG-CSF is routinely administered to volunteer donors for granulocyte transfusions.4

The development of nonmyeloablative and reduced-intensity preparative regimens is extending the use of allogeneic PBPC transplantation to older patients, whose siblings are approximately the same age.5 The safety of rhG-CSF–induced PBPC mobilization and collection in older donors has not been well studied, and there may be increased potential for unexpected and possibly deleterious short-term biologic and molecular effects. This review will summarize recent data regarding these effects of rhG-CSF in healthy donors, as well as the new and unique set of challenges they create.

Physiologic versus pharmacologic exposure to G-CSF

Any concern involving the pharmacologic administration of rhG-CSF to healthy subjects needs to recognize that G-CSF is a naturally occurring cytokine, whose levels are known to be significantly increased in healthy individuals in response to physiologic stimuli, primarily infection. Whether the physiologic neutrophilia of pregnancy is associated with a rise in plasma G-CSF levels remains controversial.6

In one study,7 the serum G-CSF level in healthy volunteers was found to be 25.3 (± 19.7) pg/mL, with no detectable difference due to age or sex. The normal range is considered to be less than 78 pg/mL.8 The serum G-CSF level in the acute phase of respiratory or urinary tract infection was 731.8 (± 895) pg/mL, roughly 30 times higher.7 Serum G-CSF levels were found to be elevated (approximately 10-fold) in bacterial but not in viral infections or infections caused by Mycoplasma pneumoniae.9 In patients with septic shock due to a variety of conditions, endogenous G-CSF serum concentrations ranged from less than 50 pg/mL up to 2885 pg/mL.10

After subcutaneous administration of a single 300-μg dose of filgrastim to 2 healthy volunteers, peak G-CSF plasma levels were observed at 4 hours after injection (25 000 pg/mL and 44 000 pg/mL), a 500- to 1000-fold increase. At 24 hours after injection, G-CSF levels were approximately 300 pg/mL, and returned to preinjection values at 48 hours.8 In a group of healthy subjects, peak serum levels of G-CSF after the subcutaneous administration of a single 300-μg filgrastim dose were 33.4 (± 7.5) ng/mL.11

Therefore, while healthy subjects can be exposed during their lifetime to supranormal endogenous G-CSF levels lasting for up to several days with no long-lasting effect, subcutaneous rhG-CSF administration to healthy donors may temporarily boost exogenous G-CSF levels well above the values usually reached under conditions such as bacterial infections.

The G-CSF receptor

The G-CSF receptor (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-p34.3.12,13

The G-CSFR is present on pluripotent and myeloid-committed progenitors, as well as differentiated myeloid cells from the myeloblast to the mature neutrophil.12,13 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 (approximately 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. Signaling pathways activated by the G-CSFR are complex and include activation of JAK tyrosine kinases as well as STAT transcription factors.13 The binding of G-CSF increases proliferation and enhances the function of myeloid cells. G-CSFR expression is down-modulated by exposure to endogenous G-CSF.12,13

The presence of G-CSFRs is not restricted to myeloid cells as originally thought, although the biologic effects of G-CSFR expression in nonmyeloid 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 and glial cells as well.12,14-17 These findings have potential implications for healthy donors, and the role and functional significance of these receptors in nonmyeloid tissues require further study.

The G-CSFR structure has been studied in detail, particularly in the contest of severe congenital neutropenia (SCN).13,18,19 SCN represents a heterogeneous group of disorders, and it is frequently caused by heterozygous germ line mutations in the ELA2 gene encoding neutrophil elastase. It carries a significant risk for evolution to myelodysplasia (MDS) and acute myeloid leukemia (AML).13,18,21

Nonsense point mutations in the G-CSFR gene were initially reported as causative of at least some SCN cases. They are currently believed to be acquired, nonheritable somatic events detected in the process of evolution to MDS/AML and therefore define a subgroup of SCN patients with increased susceptibility to MDS/AML.18-21 The mutations result in the truncation of the G-CSFR C-terminal cytoplasmic region, and disrupt its granulocytic maturation signaling function.6,21 They lead to an abnormally high proliferative response to G-CSF in cultured myeloid cells. This may lead to excess G-CSF stimulation and the accumulation of immature cells that do not differentiate, hence accounting for the higher risk of AML/ MDS development.22

While the risk of MDS/AML in SCN increases with the duration of rhG-CSF therapy,18,19 this may simply reflect the improved survival of these patients, which allows the evolution to MDS/AML to occur. The exact role of rhG-CSF administration in this process remains controversial, and the implications for its use in healthy donors, if any, are unclear.

**Hematologic effects of rhG-CSF**

**Neutrophil kinetics and functional status**

RhG-CSF–induced effects on neutrophils are well described.1,8,23 G-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. G-CSF activates neutrophils enhancing their phagocytic function, including respiratory burst metabolism, surface CD11b/CD18 antigen expression and cellular elastase activity.1,8,23

**Monocytes**

A 5-day course of rhG-CSF will lead, on average, to a 3-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.24 There is evidence for the presence of G-CSF receptors on at least some monocyte subsets.14,25 Monocytes from rhG-CSF–treated healthy subjects produce more IL-10 than unmobilized monocytes in response to proinflammatory factors such as lipopolysaccharide (LPS).25 Fraser et al26 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+/CD14low/white), and the ability of these cells to stimulate alloreactivity was blunted.

**Platelets and coagulation**

Data on the effects of rhG-CSF on platelet function in healthy 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 al16 identified functional G-CSF receptors on platelets. They also found increased ADP-induced platelet aggregation in 4 rhG-CSF–treated healthy volunteers.27 In one study, rhG-CSF administration caused increased ADP-induced platelet aggregation and enhanced functional platelet activity in vitro.28 However, the assays reported in this study and the methodology used have not been subsequently validated by other investigators, and the results have not been reproduced. Another, more recent study using a more validated and accepted methodology found reduced platelet aggregation.29 No firm conclusion can be drawn at present on the effect of rhG-CSF administration on platelet function.

The impact of rhG-CSF treatment on the coagulation system in healthy donors has been evaluated in more detail. Falanga et al23 reported an impact of rhG-CSF on hemostasis in healthy 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 endothoxin-induced mononuclear cell procoagulant activity. These changes were largely resolved 1 week after stopping treatment. Topcuoglu et al30 reported similar findings. Leblanc et al26 found increased levels of factor VIII:C and thrombin generation in healthy donors after rhG-CSF administration. Söhngen et al31 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 healthy donors, although the clinical relevance of this remains unclear. Rare donors with vascular events have been reported during or shortly after PBPC donation.32

The effects of rhG-CSF on platelet function and the coagulation system are of particular interest, in view of the ongoing regenerative medicine studies. rhG-CSF has been proposed as an agent to mobilize endogenous stem cells for tissue regeneration. This approach has been studied in healthy subjects with active coronary artery disease (CAD) and myocardial infarction.33,35 The effects of platelet activation and hypercoagulability could prove deleterious in this setting. In a study of rhG-CSF administration in 15 patients with CAD, 2 experienced a myocardial infarction (MI), seemingly temporally related to rhG-CSF administration.35 Kang et al34 reported a significant increase in in-stent restenosis in rhG-CSF–treated MI patients after percutaneous coronary intervention. In
another study the incidence of angiographic restenosis was not affected by rhG-CSF. In view of these findings, caution should be exercised with rhG-CSF administration in subjects with known or suspected active CAD, as well as older PBPC donors with cardiovascular disease.

**Immunologic effects of rhG-CSF**

**Lymphocytes**

The presence of G-CSFRs on lymphocytes has been controversial. Franzke et al detected G-CSFR expression in class I and class II-restricted T cells both in vivo and in vitro in healthy 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 up-regulation of surface activation markers (CD69 and CD53) and transcription factors (GATA-3), accompanied by down-regulation of costimulatory (CD5 and CD44) and adhesion (LFA-1x) molecules. These effects may be indirect. Boneberg et al 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 healthy donors. Lindemann et al showed that rhG-CSF administration to healthy donors suppresses cellular immune function within days and increases soluble HLA antigen levels. These effects resolved by 2 months of treatment. In another study, donor treatment with pegylated G-CSF augmented the generation of IL-10-producing regulatory T cells. 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.

**Natural killer cells**

One study showed no effect of rhG-CSF administration on the PB natural killer (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 healthy donors outside the myeloid lineage may be caused by the transient modulation of cytokine responses and networks. Weiss et al 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 proinflammatory and anti-inflammatory effects.

Boneberg et al 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 showed that T-cell exposure to rhG-CSF is associated with spontaneous IL-4 release. Lindemann et al have shown a significant rise in IL-10 plasma levels in volunteer PBPC donors. A surge in IL-8 serum levels after rhG-CSF administration was described by Watanabe et al.

**Effects on cell mobilization**

**PBPCs**

RhG-CSF ability to mobilize PBPCs in healthy donors has been studied in detail and the mechanism(s) involved recently reviewed. 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. Adhesion molecule shedding is believed to occur with mobilization, and 2 studies in healthy donors have shown increased serum levels of soluble adhesion molecules (sL-selectin, sE-selectin, sCD44 but not sCAM-1) after rhG-CSF administration.

**Dendritic cells**

Another recently established effect of rhG-CSF in PBPC donors is represented by its ability to mobilize dendritic cells (DCs). Mature DCs are antigen-presenting cells involved in the cellular immune response, and have a unique ability to stimulate naive T cells. Two separate lineages of DCs have been described in humans (DC1 and DC2), according to their ability to trigger naive T-cell differentiation to Th1 and Th2 cells, respectively. 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.

Arpinati et al studied rhG-CSF–treated healthy donors, as well as healthy controls. While the rhG-CSF–treated donors were not used 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 studied rhG-CSF–treated healthy volunteers, used as their own controls. They found that rhG-CSF only increased ( > 7-fold) the plasmacytoid T cell–derived CD11c− DC subset. It should be emphasized that both of these studies were small and further validation of these data will be required.

**Endothelial progenitor cells**

rhG-CSF also mobilizes endothelial progenitor cells (EPCs), which may have significant implications for tissue regeneration and angiogenesis. 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+CD133+VEGFR-2+ EPCs increased 8-fold from steady state, and the concentration of CD34+, CD133−VEGFR-2+ EPCs increased 10-fold. While this study is provocative, it is a small one (8 donors) and cell subsets were defined only based on surface markers, not functional characteristics. Allan et al looked at vascular progenitor cells (VPCs) mobilization in healthy donors (n = 21) as well as in patients receiving cyclophosphamide plus G-CSF. They used functional assays, and found that VPC mobilization occurred independently of hematopoietic progenitor mobilization.

These are preliminary data generated in small studies and further research will be required to clarify the role of rhG-CSF as EPC mobilizer in healthy subjects. Interestingly, in a study...
involving CAD patients, rhG-CSF–induced EPC mobilization was found to be reduced compared with healthy controls.51

Effects on chromosomal integrity

Arguably the most provocative recent finding related to rhG-CSF administration in healthy individuals has been a possible effect on chromosomal integrity. Nagler et al52 studied PB lymphocytes in rhG-CSF-mobilized donors (as well as 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 9 months after rhG-CSF exposure, and was felt to be related to changes in DNA methylation capacity. This study has generated considerable interest and concern worldwide. However, several questions have been raised on the study methodology and data analysis used. 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 used, some lack of clarity around the total dataset from which the presented data were extracted as well as the use of a nonconventional statistical significance level.

Kaplinsky et al53 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 with the possibility of a selection bias, as well as the lack of data on untreated controls.

It should be emphasized that these studies are small, have been subject to criticism, have not been replicated so far, and should be viewed as inconclusive. Even if reproduced, any clinical correlation of the development of chromosomal abnormalities with the actual risk of developing a hematologic malignancy would be unclear. A parallel could be drawn with the presence of B cells carrying the t(14;18) translocation (observed in most patients with follicular lymphoma) in the peripheral blood of healthy individuals.54

To address the concerns raised by these studies, the National Marrow Donor program (NMDP) has recently 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 US incidence of cancer in the adult population, with no reports of leukemia or lymphoma.55 The robust sample size for these data, as well as the adequate follow-up provide significant reassurance, although they do not eliminate the need for continued surveillance.

In addition, the United Kingdom Donor Registries have proposed a study to screen rhG-CSF–treated healthy donors for secondary FISH on the same cells with autosomal probes. The presence of CD34+ 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 with the possibility of a selection bias, as well as the lack of data on untreated controls.

Effects on gene-expression patterns

While data in this area remain sketchy and not widely reproduced so far, 2 groups,57,58 have reported on the transient modulation of expression for hundreds of genes in healthy subjects receiving rhG-CSF as studied by gene microarray technology. A study on 9 healthy donors57 showed up-regulation of multiple genes involved in neutrophil activation, as well as down-regulation 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 2 months. In a similar study,58 approximately 300 genes exhibited changes in the level of expression, with gradual normalization over 6 months. Fifty-three genes involved in cell growth, proliferation, and communication gene ontology categories were up-regulated (including the gene encoding for the CD34 antigen), while 69 genes were down-regulated (including the gene encoding the CCAAT/enhancer binding protein ε, a transcription factor). Shapira et al59 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 to 2 months. It should be noted that in all of these studies the small sample size, the lack of appropriate controls and specific information on the validation of the assays used should prompt caution in the interpretation of the results.

Effects on bone metabolism

rhG-CSF affects bone metabolism in healthy subjects.60,61 rhG-CSF has been shown to cause a transient enhancement of osteoclastic activity, as well as short-term inhibition of bone formation. Bone-specific alkaline phosphatase serum levels are transiently increased, while osteocalcin levels are decreased.60,61 PB mononuclear cells of rhG-CSF–treated healthy donors have increased osteoclastogenic potential.62

Effects on nonhematopoietic growth factors

rhG-CSF causes an increase in the serum levels of vascular-endothelial growth factor (VEGF) and, even more convincingly, hepatocyte growth factor (HGF) in healthy donors.63 rhG-CSF–treated donors also have increased plasma levels of transforming growth factor beta (TGF-β).64

Conclusions

As more healthy donors are exposed to pharmacologic doses of rhG-CSF, it has become essential to study its effects to safeguard donor safety. Accumulating evidence now suggests that rhG-CSF effects in healthy subjects may be more complex and heterogeneous that originally thought. They may well go
beyond a selective impact on the myeloid series and involve other cell types and tissues. The vast majority of these effects are transient, self-limiting and unlikely to cause significant concerns. 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, more durable rhG-CSF–induced impact on chromosomal integrity has been raised on the basis of limited, preliminary, and largely uncontrolled data, and will need further evaluation and prospective studies. Available data have not indicated an increase in malignancies in PBPC donors, but continued surveillance is clearly warranted. Based on the totality of information that is currently available, we believe that the administration of rhG-CSF to healthy donors for the purpose of PBPC donation continues to have a favorable risk-benefit profile.

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