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THE ADMINISTRATION OF GRANULOCYTE COLONY-STIMULATING FACTOR TO HEALTHY DONORS FOR ALLOGENEIC PERIPHERAL BLOOD PROGENITOR CELL COLLECTION MAY INDUCE THE TISSUE FACTOR DEPENDENT PATHWAY

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SUMMARY

The hypercoagulable state caused by the use of rhG-CSF has been cited in several case reports. Since tissue factor (TF) is the main initiator of the coagulation protease cascade, we examined whether or not rhG-CSF had an inductive effect on the TF-dependent pathway. We measured plasma TF antigen (TF Ag) and TF procoagulant activity (TF PCA), TF expression on peripheral blood monocytes and neutrophils and plasma coagulation factor activities in 18 healthy donors (8F/10M; aged 17-52) receiving 10μg/kg/day rhG-CSF for five days in the aim of peripheral blood progenitor cell mobilization. Blood samples were collected before starting G-CSF and on the first day of stem cell apheresis. There were significant Increases in TF Ag ($p<0.05$) and TF PCA ($p=0.06$) levels. Flow cytometric evaluation revealed a significant increase in TF expression on CD33 (+) cells ($p=0.04$). Mean plasma FVIII activity and vWF activity also increased significantly. Thrombin time was slightly prolonged ($p=0.06$) due to significant increases in plasma D-dimer levels ($p<0.05$). In addition, while FIX activity remained stable, there were marked decreases in mean plasma FX and FII activities and a slight decrease in FVII activity that resulted in significant prolongation of prothrombin time. The administration of rhG-CSF in healthy stem cell donors increased the mean TF Ag and TF PCA in plasma and TF expression on cells, decreased extrinsic pathway factor activity, increased D-dimer levels and endothelial markers and prolonged PT. In conclusion, the administration of rhG-CSF led to a 'prothrombotic state' via stimulation of TF and increased endothelial markers such as F VIII and vWF. In light of these findings, the use of rhG-CSF for stem cell mobilization should be undertaken cautiously in healthy donors with underlying thrombotic risk factors.

Key Words: *Coagulation, G-CSF, Healthy Donor, Tissue Factor*

ÖZET

ALLOJENEİK PERİFERİK KÖK HÜCRE TOPLANAN SAĞLIKLI VERİCİLERDE GRANÜLOSİT KOLONİ STİMÜLE EDİCİ FAKTÖR KULLANIMI DOKU FAKTÖRÜ BAĞIMLI YOLU UYARABİLİR

Granülosit koloni stimule edici faktör (rhG-CSF) kullanımının neden olduğu hiperkoagulabiliteden değişik vaka sunularında söz edilmektedir. Doku faktörünün (DF) koagulasyon-proteaz kaskadının esas başlatıcısı olduğundan dolayı, rhG-CSF'nin DF bağımlı yol üzerine uyarıcı bir etkisi olup olmadığı çalışıldı. Periferik kan kök hücre mobilizasyonu amacı ile 10μg/kg/gün rhG-CSF 5 gün süre ile kullanan 18 sağlıklı (8K/10E, yaş 17-52) vericide, plazma DF antijen (DF:Ag), DF prokoagulan aktivite (DF:PKA), periferik kandaki monosit ve nötrofillerde DF:Ag ekspresyonu ve plazma koagulasyon faktör aktivite düzeyleri çalışıldı. Kan örnekleri G-CSF başlamadan önce ve kök hücre aferezinin ilk günü toplandı. DF:Ag ($p<0,05$) ve DF:PKA'da ($p=0,06$) önemli artış vardı. Akım sitometrik değerlendirme CD33 (+) hücrelerde DF ekspresyonunda anlamlı bir artış ortaya kondu ($p=0,04$). Beraberinde plasma FVIII ve vWF aktivitesi de anlamlı olarak arttı. Trombin zamanı, plazma D-Dimer seviyesindeki anlamlı artış ($p<0,05$) nedeni ile hafif uzadı ($p=0,06$). Ek olarak FIX aktivitesi sabit kalırken, ortalama plazma FX ve FII aktivitelerinde ise belirgin azalma ve protrombin zamanında (PTZ) anlamlı uzamaya neden olan FVII aktivitesinde hafif bir azalma vardı. Sağlıklı kök hücre vericilerinde rhG-CSF kullanımı ortalama plazma DF:Ag ve DF:PKA ve hücrelerde DF ekspresyonunu arttırdı, ekstrensek yol faktör aktivitesini azalttı D-Dimer düzeyleri ve endotelyal belirleyicileri arttırdı ve PTZ'yi uzattı. Sonuç olarak rhG-CSF kullanımı DF uyarımı ve, FVIII ve vWF gibi endotelyal belirleyicileri arttırarak bir 'protrombotik durum''a neden olmaktadır. Bu bulguların ışığında kök hücre mobilizasyonu için rhG-CSF kullanırken sağlıklı vericilerde altta yatabilecek trombotik risk faktörleri dikkate alınmalıdır. Biz G-CSF kullanımından önce sağlıklı vericilerin altta yatan trombotik risk faktörleri yönünden değerlendirilmelerini öneriyoruz.

Anahtar Kelimeler: *Koagulasyon, G-CSF, Sağlıklı Verici, Doku Faktörü*

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Granulocyte-colony stimulating factor (G-CSF) is a hematopoietic cytokine that stimulates neutrophil production and the release of mature granulocytes from bone marrow and enhances neutrophil function (1). Because of its ability to induce mobilization of CD34+ hematopoietic progenitor cells (HPC) into circulating blood, G-CSF has recently been used in normal subjects to mobilize and collect HPC for allogeneic transplantation (1) The most commonly used protocol for stem-cell mobilisation is the administration of G-CSF to donors at a moderate-high dose such as 10μg/kg/d for four or five days and subsequent leukapheresis (1,2).

The safety of G-CSF administration in healthy donors has been investigated by several authors (1,2). However, there is limited knowledge about the effects of G-CSF on hemostasis. Thrombosis was the first event attributable to G-CSF (3), and two cases of healthy donors with acute arterial thrombosis, which might be related to G-CSF, have been documented (2). In a meta-analysis of studies investigating the use of G-CSF with chemotherapy, 1.2% of cancer patients were reported to have encountered thrombotic complications (4). Whether G-CSF has a direct role on thrombus formation is still unknown. Following preliminary studies that showed the presence of a receptor for G-CSF on platelets and megakaryocytes (5), it was claimed that G-CSF caused thrombosis by increasing platelet aggregation (6). Tissue factor (TF) is a main physiological initiator of blood coagulation-protease cascade in normal hemostasis (7). It has a cofactor role for factor VII/factor VIIa (FVII/FVIIa) during activation of factor IX (FIX) and factor X (FX) by FVII (4). TF is constitutively expressed on cells separated from the blood stream (8). Normally, peripheral blood cells (monocytes/macrophages) and endothelial cells do not express TF. However, recent studies have shown that monocytes and macrophages also contributed to thrombin generation under various pathological conditions in which TF activation on these cells increased procoagulant activity (9,10).

To date, there is a lack of knowledge about the role of TF in the hypercoagulable state induced by the use of G-CSF for allogeneic HPC mobilisation in healthy donors. This study investigated the effect of G-CSF on plasma TF antigen and TF procoagulant activity as well as TF antigen expression on peripheral blood cells, the factors involved in intrinsic and extrinsic coagulation pathways, naturally occurring anticoagulants and contribution of endothelium-derived factors in healthy donors.

MATERIAL AND METHOD

Eighteen HLA-identical healthy sibling donors who were candidates for allogeneic stem cell collection (8 female, 10 male; mean age: 32) and 18 normal control cases (no G-CSF) were studied. Donors received G-CSF (Filgrastim; Amgen, Roche) 5μg/kg subcutaneously twice daily for nine consecutive doses. Following the ninth dose on Day 5, leukapheresis was performed using a continuous-flow cell separator (COBE Spectra or Baxter CS 3000 plus).

Blood Collection: Thirty-five mLs of blood BF were collected from each donor before the first dose and after the ninth dose of G-CSF. EDTA anticoagulated tubes were used for complete blood count (CBC), differential leukocyte count (DLC) and detection of TF antigen (TF:Ag) expression. Citrated (3.8%) tubes were used for coagulation tests and of TF:Ag and procoagulant analysis activity (TF:PCA). Plasma samples obtained by centrifugation of citrated tubes (3000G, 10 min, 22°C) were processed immediately or stored at -80°C as aliquots until tests could be performed.

Assays: Plasma TF:Ag was determined using an Imubind Tissue Factor ELISA Kit (#845, BF American Diagnostic, Greenwich, USA). The kit employs a murine anti-human tissue factor monoclonal antibody for antigen capture. Prediluted plasma samples (1/10 in sample buffer) were incubated in micro test wells precoated with capture antibody. Captured TF was detected with a biotinylated antibody fragment that specifically recognised bound TF. After an incubation step using streptavidin conjugated horseradish peroxidase (HRP), the addition of TMB substrate creat-

ed a blue colour in the wells. Addition of 0.05 M sulphuric acid top solution caused it to turn yellow and stabilise. TF levels were determined by measuring absorbency at 405 nm and comparing the values with those of the standard curve.

Plasma TF:PCA was determined using an Actichrome Tissue Factor Activity Kit (#846, American Diagnostic, Greenwich, USA), which measures intact TF as well as TF/Factor VII (TF/FVII) and TF/factor VIIa (TF/FVIIa) complexes. Peptidyl activity of TF present in plasma was measured to form a complex with FVII. Plasma samples diluted 1/10 with 5% TF/Tissue Factor Pathway Inhibitor depleted plasma, mixed with human factor VIIa and incubated, allowing formation of the TF/FVII complex. The complex was allosterically activated, and its activity was directly measured by its ability to cleave to a highly specific chromogenic substrate for TF/FVIIa complexes (Spectrozyme FVIIa) that was added to the reaction solution. The cleavage of the substrate was terminated by releasing a paranitroaniline (pNA) chromophore into the reaction solution. The absorbency of the solution was read at 450 nm and compared to the values obtained from a standard curve generated using known amounts of lipidated TF. To obtain the actual TF concentration (ng/mL), results were multiplied by 10 (dilution factor) and 42 (conversion factor, 1 ng/mL TF:PCA is equal to 42 nM).

TF:Ag expression was detected using a Fluorescein IsothioCyanete (FITC) conjugated murine monoclonal antibody specific to human tissue factor (#4508CJ, American Diagnostica, Greenwich, USA). Whole blood was diluted with PBS to obtain 0.5x106 WBC/mL. In order to identify specific bounding to monocyte/neutrophils, monoclonal antibodies (moAbs) specific to CD14, CD45 and CD33 and an isotypic control were used simultaneously (Becten Dickinson Immunologic System = BDIS, San Jose, USA). Five µl of anti-TF Mo.Ab. and 7 µL of other MoAbs were added to prelabeled tubes. Monoclonal antibodies were incubated with 100 µL diluted blood for 30 min at 4(C. Following red cell lyses procedure, cells were washed twice with PBS and fixed with 500 mcL 1% paraformaldehyde solution (Cellfix, BDIS, Sana Jose, USA). Data was collected within 24 hour by counting 10.000 cells per tube in a flow cytometer (FacSort, BDIS, San Jose, USA). Data was analysed using Cell Quest Software (BDIS). Monocytes and neutrophils were gated according to forward- and side-scatter characteristics and CD33 or CD14 expression intensity. CD14+/CD33bright cells and CD33dim cells were evaluated respectively as monocytes and neutrophils. Isotypic control tubes and lymphocyte gates were used to set a marker for calculating TF expreszing cell percentage.

Other Parameters

Complete blood counts (CBC) and white blood cell (WBC) differential counts were obtained by processing anticoagulated blood specimens through a properly calibrated and quality-controlled automated hematology analyser (STKS, Coulter Beckman).

Coagulation parameters including prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), factor II (FII), FVII, FVIII, FIX, FX and plasma fibrinogen level were measured nephelometrically in an automated and quality-controlled coagulometer (ACL, Futura, IL) using reagents purchased by the same manufacturer. The level of D-Dimer was measured using a latex-enhanced turbidometric immunoassay in the same coagulometer. vWF Ri:CoF activity was measured semi-quantitatively using von Willebrand reagent (Dade Behring). Natural anticoagulants including protein C, protein S and antithrombin III (ATIII) were measured functionally.

RESULTS

Baseline laboratory parameters (Day 0) of all subjects were found to be within normal sauges (Table 1 and Table 2). Significant increases in WBC, neutrophil and monocyte counts were observed in all donors administered rhG-CSF (Day 5 vs Day 0, $p<0.001$). There were no changes in red blood cell (RBC) and platelet counts at Day 5 vs Day 0 (Table I).

Hemostatic parameters

As shown in Table I, PT measures were slightly but statistically prolonged from 11.5±0.6 sec to 12.0±0.6 sec after G-CSF ($p<0.05$). We observed a significant rise in D-Dimer levels, with a slightly prolonged TT at Day 5. G-CSF caused no change on PTT and fibrinogen levels.

As shown in Table 2, a significant rise was observed in mean activity of vWF:Ri CoF and VIII, from 70±24% to 90±20% ($p<.05$) and from 131±47% to 198±63% ($p<0.05$), respectively. However, mean activity of FII and FX decreased significantly after G-CSF, from 108±14% to 97±16% ($p<0.05$) and from 118±20% to 103±15 ($p<0.05$), respectively. The mean activity of natural anticoagulants protein C, protein S and ATIII decreased after G-CSF; however, only the decrease in protein C activity was slightly significant. There was no observable change in APCR-R after G-CSF.

Table 1: Laboratory parameters before G-SCF (Day 0) and after G-CSF (Day 5)

Variables	Day 0 (mean±SD)	Day 5 (mean±SD)	p
Leukocyte (10^9/L)	7.3±1.0	47.4±13.8	<0.001
Neutrophil (10^9/L)	4.3±0.9	40.5±13.2	<0.001
Monocyte (10^9/L)	0.4±0.1	2.1±1.7	<0.001
Red blood cell (101^2/L)	4.9±0.5	4.7±0.5	0.35
Platelet (10^9/L)	273±86	271±56	0.94
PT (second)	11.5±0.6	12.0±3.7	0.01
PTT (second)	31.5±3.7	31.4±8.1	0.96
TT (second)	12±1.0	14±2.4	0.06
Fibrinogen (mg/dL)	339±90.1	377±72.2	0.17
D-Dimer (ng/mL)	125±92.1	188±88.0	0.04

Table 2: Plasma factor activities and natural anticoagulants of healthy donors before (Day 0) and after G-CSF (Day 5)

Variables	Day 0 (mean±SD)	Day 5 (mean±SD)	P
Factor II (%)	108±14.1	97±16.0	0.03
Factor VII (%)	126±32.7	114±63.7	0.49
Factor VIII (%)	131±47.6	198±63.7	0.001
Factor IX (%)	136±44.8	157±75.0	0.32
Factor X (%)	118±20.8	103±15.5	0.02
vWF:Ri CoF (%)	70±24.0	90±20.4	0.01
ATIII (%)	108±20.5	99±19.4	0.17
Protein C (%)	106±28.4	95±8.6	0.09
Protein S (%)	100±30.4	92±28.2	0.42
APC-R	2.1±0.3	2.0±0.3	0.68

Tissue factor antigen (ELISA assay)

There was a significant increase in mean plasma TF:Ag level from 34.3±52 pg/mL (0-185 pg/mL) to 100.4±90 pg/mL (0-287 pg/mL), ($p<0.01$) while mean TF:Ag level was 100.8±78 pg/mLin the control group (Figure 1). When evaluated according to gender, the increase in mean TF:Ag level was significantly higher in males (from 37.2±58 pg/mL to 122.8±105 pg/mL, $p<0.04$) than in females (from 30.4±47 pg/mL to 70.9±72 pg/mL, $p=0.2$) (Table 3).

Tissue factor procoagulant activity (ELISA assay)

Plasma TF:PCA level increased from 9.1±20 ng/mL to 48.9±81ng/mL after G-CSF, with a tendency to be significant ($p=0.06$) (Figure 2). Although this increase was more pronounced in males, there were no significant changes in either gender (12.5±27 ng/mL to 62.3±92 ng/mL, $p=0.14$ in males; 5.2±7 ng/mL to 33.9±71 ng/mL, $p=0.27$ in females) (Table 3).

No correlation between TF:Ag and TF:PCA either before or after G-CSF could be detected.

Flow cytometrical data (Figure 3)

There was no change in TF:Ag expression on CD14+ (monocytic) cells at Day 5 vs Day 0 (16.1±23% v.s.12.8±18%, $p=0.37$). However, a significant increase in the level of TF:Ag expression on CD33+ cells after G-CSF (from 9.3±13% to 15.6±18%, $p=0.04$) was observed. This increase was detected on both CD33bright cells and CD33dim cells, from 13.2±22% to 30.1±32% ($p=0.04$) and from 3.9±8% to 19.3±25% ($p=0.01$), respectively.

DISCUSSION

To provide further insight into the action of G-CSF on hemostasis and thrombosis, we evaluated coagulation parameters of 18 healthy stem-cell donors and compared them with a control group. The mean activities of FII and FX were reduced by a ratio of 11-13% following G-CSF. Mean FVII activity also decreased slightly, resulting in prolonged PT. However, there was no change in mean FIX activity or mean PTT on Day 5. This indicates that the coagulation factors in the extrinsic pathway are consumed rather than vitamin K-dependent factors. If G-CSF had affected vitamin K-dependent factors, we would also have observed a decrease in FIX activity and prolonged PTT. To date, there has been insufficient data about the effect of G-CSF on plasma coagulation factors. G-CSF has short- and long-term effects on hemostatic parameters, other than an increase in FVIII levels following G-CSF reported by LeBlanc (11). Following intravenous G-CSF administration to healthy donors, platelet aggregation response to adenosine diphosphate (ADP) and collagen increased while D-dimer levels remained unchanged (12). In our study, we found that the level of D-dimer, an indicator of an activated coagulation system, increased significantly from 125 ng/mL to 188 ng/mL ($p<0.05$), although values were within the normal control range. Additionally, TT was slightly prolonged ($p=0.06$), possibly as a result of increased fibrinogen

Table 3: Plasma TF:Ag and TF:PCA levels before G-CSF (Day 0) and after G- CSF (Day 5)

Variables	Day 0 (mean±SD)	Day 5 (mean±SD)	P
TF:Ag (pg/mL) (n=18)	34.3±52	100.4±92	0.01
Female (pg/mL) (n=8)	30.4±47	70.9±72	0.20
Male (pg/mL) (n=10)	37.2±58	122.8±105	0.04
TF:PCA (ng/mL) (n=18)	9.1±20	48.9±81	0.06
Female (ng/mL) (n=8)	5.2±7	33.9±71	0.27
Male (ng/mL) (n=10)	12.5±27	62.3±91	0.14

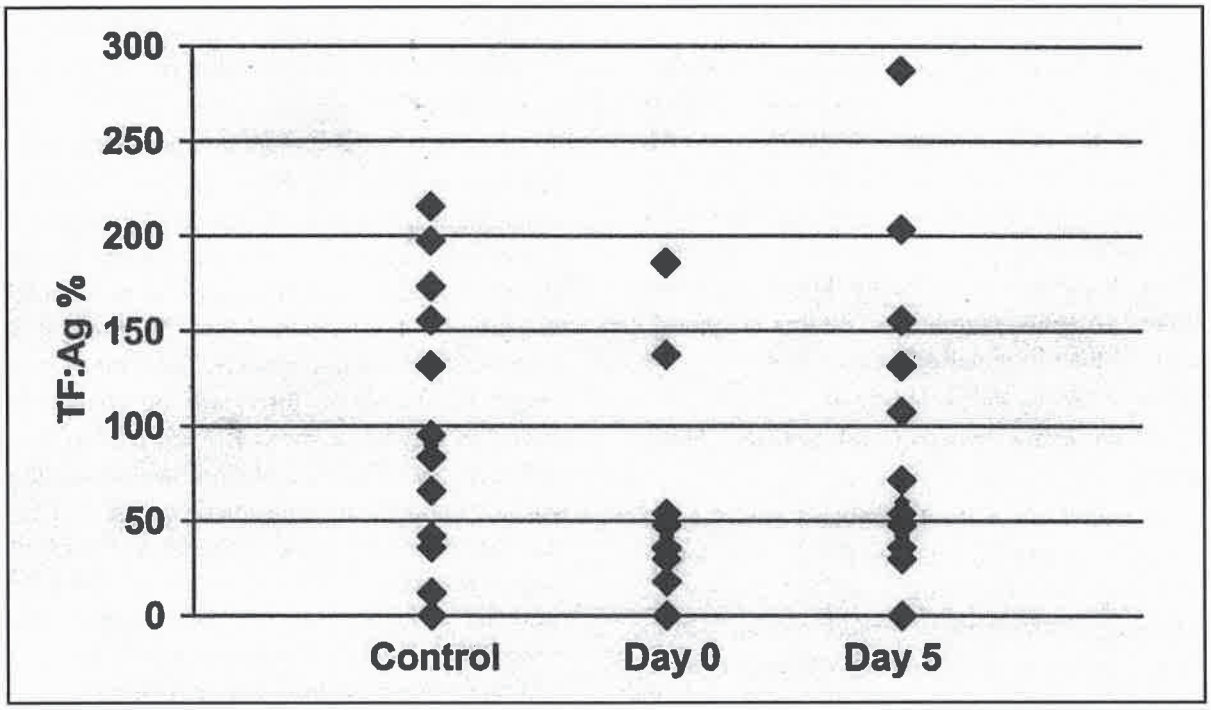

Figure 1: TF:Ag (pg/mL) levels in control group and healthy donors

turnover. Falanga et al reported a similar increase in D-dimer levels, although within normal control range, at the first day of apheresis, using a higher dose of G-CSF (15μg/kg/day) (10). Our study presents the first line of evidence that the coagulation cascade is triggered following G-CSF

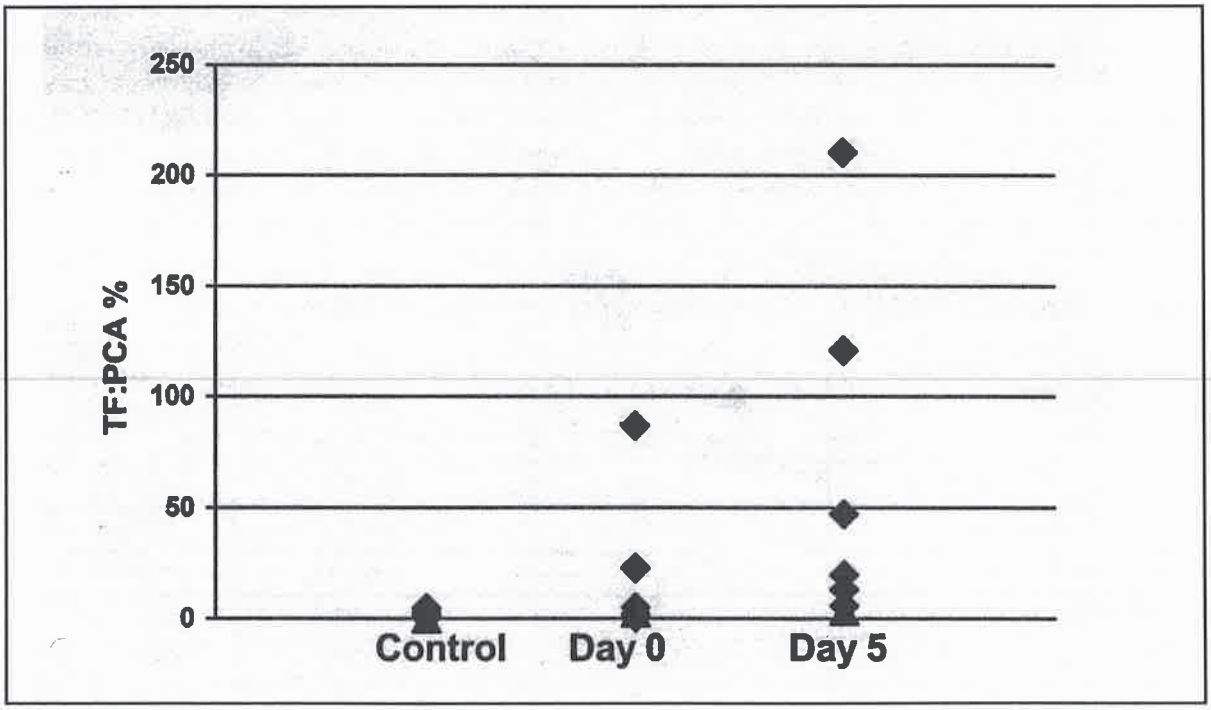

Figure 2: TF:PCA (ng/mL) levels in control group and healthy donors

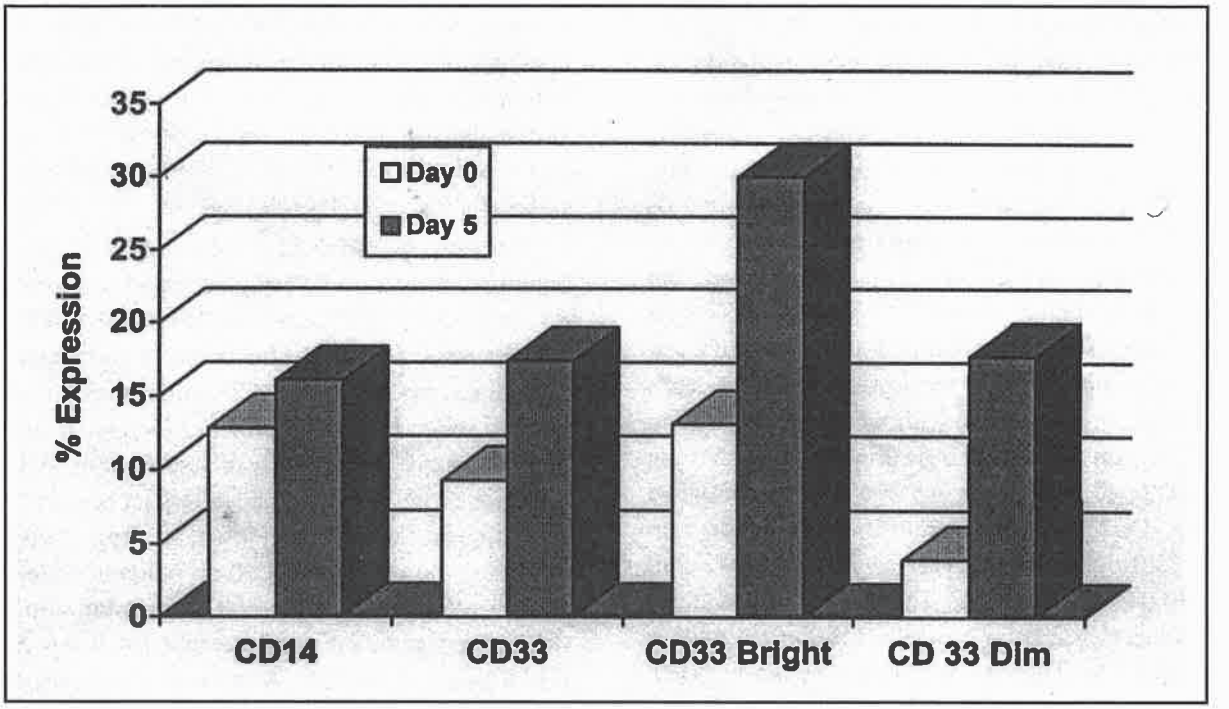

Figure 3: Comparison of mean TF:Ag expression (flow cytometric) on peripheral blood monocytes and neutrophils before rhG-CSF (Day 0) and after rhG-CSF (Day 5)

administration by demonstrating a decrease in coagulation factor activity of extrinsic pathway, prolongation of PT and elevation of D-dimer levels.

At present, our knowledge about the contribution of growth factors on TF and/or TF-dependent pathway of coagulation is limited. We observed significant increases in the mean levels of both TF:Ag and TF:PCA in plasma after G-CSF ($p=0.01$ and $p=0.06$, respectively). However, there was no correlation between TF:Ag and TF:PCA at either Day 0 or Day 5. One of the possible reasons for this conflict may be the incomplete activation of TF:Ag. It has been suggested that with respect to procoagulant activity, the majority of cell surface TF activity is normally "encrypted", in which state it is capable of binding antibody and FVII/VIIa, but not fully expressing antigenic activity. To become fully activated, it must be "de-encrypted" by processes involving plasma membrane phospholipids and monomerization of TF (13). In previous reports, G-CSF was shown to increase monocyte procoagulant activity (10). No influence of age and gender on plasma level of TF:Ag has been demonstrated in normal individuals (14). In our study, the elevation of TF:Ag after G-CSF tended to be more pronounced in males (from 37pg/mL to 123pg/mL, $p<0.05$) than females (from 30.4pg/mL to 71pg/mL, $p=0.2$) (Table 3). Data related to the role of TF in the development of atherosclerosis and an increased incidence in men must be evaluated within this context.

In normal individuals, TF:PCA in the circulation is very low (14). What is the source of TF:Ag and TF:PCA after G-CSF is a question that should be answered. While there is debate over the contribution of neutrophils to TF:PCA (15,16), the main sources of TF in the circulation are considered to be monocytes and macrophages (9). It has been put forward that circulating endothelial cells might have been contributing to TF:PCA during the aplasia period of stem-cell transplantation because of the absence of mature myelo-

monocytes (17). However, Özcan et al followed TF:PCA of stem cell transplant recipients during the peritransplant period and detected a considerable amount of remaining TF:PCA originating neither from myelo-monocytes nor circulating endothelial cells (18). The rise of endogenous G-CSF levels during the aplasia period of stem-cell transplantation has been previously shown, but the effect of endogenous G-CSF levels on TF:PCA remains to be elucidated (19). We observed a significant increase in TF expression on CD33+ cells (mature myeloid cells) without a major change in TF:Ag on CD14+ cells (monocytes). Among CD33+ cells, the increase was more pronounced on CD33dim (neutrophil) cells than on CD33bright (monocyte) cells ($p=0.01$ and $p=0.04$, respectively). These two observations lead us to suspect a possible contribution of neutrophils to TF:PCA. While an increase of TF expression has been shown on G-CSF-treated neutrophils in vitro (20), a clear correlation between the number of monocytes and increased TF expression has been demonstrated in vivo (16,21).

The acquisition of TF:Ag by neutrophils may have contributed to the increased TF levels in our study, and monocytes are likely to have played a role in this mechanism.

G-CSF exerts an important effect on endothelial cells by inducing their migration and proliferation (22). vWF and thrombomodulin (TM) are not only released from endothelial cells, but also accepted as surrogate markers of stimulation and toxicity of the endothelium (23). There have been some studies reporting that G-CSF increases the levels of vWF antigen, vWF activity and soluble TM (10,11). We found a significant elevation of plasma vWF:Ri CoF and FVIII activity after G-CSF. The mechanism by which G-CSF exerts its effects on the endothelium has not been fully explained. Upon activation, neutrophils release reactive oxygen species and intracellular protease that perform several activities on endothelial cells and platelets and may modify the hemostatic balance towards a prothrombotic state (10,24), to which a series of leukocyte-mediated events may have contributed, in addition to direct toxicity. As we did not investigate the relationship between levels of neutrophil-derived proteases and activation markers, we were not able to demonstrate the role of leukocyte-mediated endothelial toxicity.

The most common factor causing prothrombotic tendency is hereditarily activated protein C resistance due to FV-Leiden mutation (25). G-CSF did not result in APCR phenotype in our study. Data about the effect of G-CSF administration on natural anticoagulants is still insufficient. It has been reported that the use of G-CSF after HSC transplantation had no negative impact on natural anticoagulants (26). However, one study observing the effect of G-CSF on patients undergoing allogeneic stem-cell transplantation showed a significant decrease in ATIII levels at Day 5 versus Day 0 (27). Another study of cancer patients showed that administration of G-CSF at different dosages (1, 3, 10, 30 and 60 mcg/kg/day, iv) for 14 days did not affect ATIII levels (28). After G-CSF, we observed a mild reduction of natural anticoagulants with a marked tendency of protein C levels. In contrast, a study using higher doses of G-CSF (12.5mcg/kg/day) on 25 healthy donors showed a significant increase in protein C and protein S levels and a slight decrease in ATIII levels (29). Moreover, leukocyte-derived proteases such as elastase, which is vulnerable to G-CSF, have been known to suppress protein C, protein S and ATIII activities via proteolysis (10). The increase in activated neutrophils as a result of G-CSF might have contributed to the reduction in the activities of natural anticoagulants.

The prothrombotic state seldom encountered with the administration of G-CSF to both healthy donors and patients still needs to be clarified. Our study has provided the first evidence demonstrating that G-CSF increases the consumption of extrinsic pathway factors by stimulating coagulation by TF and the production of D-dimer, enhances vWF and FVIII levels by stimulating endothelial cells and reduces the activity of natural anticoagulants. Therefore, we propose that

before administration of G-CSF to both donors and patients with known risk factors, a survey should be undertaken for evidence of inherited thrombophilia, including FV Leiden mutation, which has a quite frequent incidence of 3-10% in the population. In individuals with underlying risk factors (hereditary and/or acquired thrombophilia), the responsible physician should take the adverse effects of G-CSF on hemostasis into consideration.

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