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Granulocyte colony-stimulating factor increases expression of adhesion receptors on endothelial cells through activation of p38 MAPK

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BERTA FUSTÉ
ROBERTO MAZZARA
GINÉS ESCOLAR
ANNA MERINO
ANTONIO ORDINAS
MARIBEL DÍAZ-RICART

A B S T R A C T

Background and Objectives. Granulocyte colony-stimulating factor (G-CSF) is specific for the granulocytic cell line, although receptors for this cytokine have been found in other cell types including endothelial cells. These observations prompted us to investigate the potential effect of G-CSF on the endothelium.

Design an Methods. Endothelial cell monolayers were exposed to G-CSF to evaluate: i) signal transduction mechanisms, ii) expression of adhesion receptors at the cell surface, and iii) leukocyte adhesion on EC monolayers.

Results. Exposure of human umbilical vein endothelial cells (EC) in culture to G-CSF resulted in the activation of the signal transduction pathways JAK/STAT (JAK-1, STAT-1 and STAT-3) and RAS/MAPK (MAPK p42/44 and p38 MAPK). We also observed significantly increased expression of the adhesion receptors, E-selectin (ELAM-1), vascular endothelial cell adhesion molecule-1 and intracelleular adhesion molecule-1 at the cell surface in response to G-CSF, increases that were followed by an augmented adhesion of leukocytes on the previously exposed EC monolayers. These effects were blocked by the presence of SB203580, a p38 MAPK inhibitor, by U0126, a MAPK p42/44 inhibitor, and by inhibiting the G-CSF receptor with a specific antibody.

Interpretation and Conclusions. Our results demonstrate that G-CSF increases the expression of adhesion receptors on EC, promoting leukocyte adhesion. This effect seems to be triggered by the signaling events that follow receptor binding. Results from experiments using specific inhibitors suggest that activation of p38 MAPK is required to promote expression of adhesion receptors in endothelial cells and the recruitment of leukocytes in response to G-CSF.

Keywords: G-CSF, endothelium, signaling, adhesion receptors, leukocyte recruitment.

From the Servicio de Hemoterapia-Hemostasia, Hospital Clínic, IDIBAPS, Barcelona, Spain.

Correspondence: Maribel Diaz-Ricart, Ph.D., Servicio de Hemoterapia-Hemostasia, Hospital Clínic Villarroel, 170, 08036 Barcelona, Spain. E-mail:mdiaz@clinic.ub.es

Granulocyte colony-stimulating factor (G-CSF) is the major growth factor responsible for regulating granulopoiesis and promoting the survival, proliferation, functional activation, and maturation of cells of the neutrophil lineage.[1] G-CSF also promotes mobilization of $CD34^+$ hematopoietic progenitor cells to circulating blood. Recombinant G-CSF is widely used to induce mobilization of blood precursors in different clinical settings such as chemotherapy-induced myelosuppression and peripheral blood stem cell collection for autologous and allogeneic bone marrow transplantation.[2]

G-CSF exerts its biological effects through interaction with specific cell surface receptors.[3] The G-CSF receptor (G-CSFR) belongs to the growth factor receptor superfamily. It has a composite structure consisting of an immunoglobulin-like domain, a cytokine receptor homologous domain, and three fibronectin type III domains in the extracellular region.[4] The G-CSFR is connected to the signaling pathways JAK/STAT[5] and RAS/MAPK.[6] There are numerous G-CSFR subclasses,[7] which differ in their cytoplasmatic sequences conferring differential signaling properties that can result in either proliferation or maturation of the neutrophil lineage. Like other cytokines, G-CSF exerts a pleiotropic effect on the different cell types presenting receptors for this growth factor.[7]

G-CSFR is expressed not only by progenitor/mature neutrophilic granulocytes but also by endothelial cells.[8] The vascular endothelium is a dynamic interface between circulating blood components and extravascular tissues. It plays an active role in maintaining the hemostatic balance, and controlling immunological and inflammatory responses. A recent report has pointed out

that endothelial cells support the expansion of stem cells *in vitro*.[9] Exposure of endothelial cells to certain physiopathologic conditions, in some of which cytokines play a significant role, may result in functional modifications, such as expression of adhesive receptors on the cells' surface. It has been reported that healthy donors treated with recombinant G-CSF to mobilize hematopoietic progenitor cells have increased levels of endothelial activation markers in blood, such as soluble forms of adhesion molecules,[10] von Willebrand factor and thrombomodulin.[11] The origin of this *in vivo* activation is difficult to elucidate since exposure to G-CSF may cause activation of other cell lineages with production of other pro-inflammatory cytokines.

In this study we explored the possible direct effect of G-CSF on cultured human umbilical endothelial cells (EC). Special attention was paid to the potential pro-inflammatory response of cells exposed to G-CSF. For this purpose, we exposed EC to G-CSF *in vitro* to analyze the signaling pathways JAK/STAT and RAS/MAPK. Modifications in the expression of the adhesion receptors E-selectin (ELAM-1), vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1) on EC were also evaluated. Moreover, to assess whether G-CSF has a pro-inflammatory effect, leukocyte adhesion on EC monolayers was asessed under static and flow conditions.

Design and Methods

Experimental design

Confluent EC monolayers[12] were incubated with increasing concentrations of G-CSF for different times. We assessed changes in: i) the intracellular signaling through tyrosine phosphorylation of proteins, ii) the expression of endothelial adhesion receptors, such as ELAM-1, VCAM-1 and ICAM-1, and iii) the adhesion of leukocytes on EC monolayers under static and flow conditions. Blocking strategies were used to confirm the specificity of our experiments. For this purpose, the effects of an antibody to the G-CSF receptor (G-CSFR) (1 μg/mL), a specific inhibitor of p38 MAPK (SB 203580, at 25 μM), and a specific inhibitor of MAPK p42/44 (U0126, at 10 μM), were evaluated.

Reagents

Recombinant human G-CSF was obtained from Amgen Europe (Breda, The Netherlands). Recombinant anti-phosphotyrosine (RC20) monoclonal antibody was from Transduction Laboratories (Lexington, KY, USA). The antibody anti-G-CSFR was from RD Systems (Minneapolis, USA). Anti-JAK-1, anti-STAT-1, anti-STAT-3, anti-phospho Map Kinase (ERK42/44) and anti-ERK p42/44 antibodies were all from Upstate Biotechnology (NY, USA), whereas anti-phospho p38 MAPK, anti- p38 MAPK, anti-phospho JNK, and anti-JNK antibodies and the inhibitor U0126, were all from Cell Signaling (New England Biolabs, UK). Monoclonal antibodies against ELAM-1, VCAM-1, and ICAM-1 came from Chemicon Int Inc. (Mississauga, Canada). Medium 199, glutamine, penicillin, and streptomycin were from Gibco BRL, Life Technologies S.A. (Barcelona, Spain). Benzamidine, EGTA, leupeptin, phenylmethylsulfonyl fluoride, pepstatin, ortho-vanadate, SB 203580, and tyrphostin 47 were from Sigma Chemical Co. (St. Louis, USA). Protein A-Sepharose was from Pierce (Rockford, IL, USA). Electrophoresis reagents and nitrocellulose membranes were from Bio-Rad Laboratories S.A. (Madrid, Spain). Enhanced chemiluminiscence (ECL) reagents, Hyperfilm-ECL, and immunogold labeling and silver enhancement were from Amersham Pharmacia Biotech Europe GmbH (Barcelona, Spain).

Evaluation of phosphotyrosine proteins

EC were starved for 24 hours before experiments were performed by replacing their growth media with media containing 0.5% pooled human sera. Confluent monolayers were incubated with different concentrations of G-CSF (0, 0.2, 2, and 20 μg/mL) for 30 min or incubated with 2 μg/mL of G-CSF for different periods of time (from 30s to 30min). EC were lysed with Laemmli's buffer (125 mM Tris-HCl, 2% SDS, 5% glycerol and 0.003% bromophenol blue) containing 2 mM sodium ortho-vanadate and 0.625 mg/mL N-ethylmaleimide, as inhibitors, under reducing conditions, subjected to SDS-PAGE and transferred onto nitrocellulose membranes.[13] Tyrosine phosphorylated proteins were detected on membranes probed with a horseradish peroxidase-conjugated anti-phosphotyrosine antibody (RC20), and developed by the ECL method.[14] Protein bands were densitometrically analyzed (Kodak Digital Science 1D, Eastman Kodak Company, Rochester, NY, USA).

Immunoprecipitation of the G-CSF receptor in endothelial cells

Endothelial cells exposed to 2 μg/mL G-CSF for different periods ranging from 30s to 30 min were lysed with modified RIPA buffer (50 mM Tris-HCL, 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate) containing phosphatase and protease inhibitors (1 mM PMSF, 1 mM EGTA, 1 μg/mL leupeptin, 1 μg/mL pepstatin, 1mM ortho-vanadate and 1mM NaF). A preclear step was also performed by adding protein A/G-agarose/sepharose beads to the sample. Aliquots of lysates containing equal amounts of protein were incubated overnight with an anti-G-CSFR, and captured with affinity purified rabbit anti-mouse IgG coupled to protein A-agarose. Additionally, samples were carefully washed 6 times with ice-cold RIPA buffer

before resuspending the immunocomplexes in Laemmli's buffer. Samples were resolved by 8% SDS-PAGE and proteins transferred to nitrocellulose membranes, which were probed with RC20 to detect the phosphotyrosine proteins associated. The presence of the blotted proteins was checked by probing the membranes with specific antibodies to the G-CSF receptor, JAK-1, STAT-1, and STAT-3.

Phosphorylation kinetics of MAPK proteins

Before and after being incubated with G-CSF (for 30s to 30 min) EC were lysed with Laemmli's buffer. Samples were resolved by 8% SDS-PAGE and proteins transferred to nitrocellulose membranes, which were probed with specific antibodies to the phosphorylated proteins: ERK p42/44 (anti-phospho Map kinase), p38 MAPK and JNK. The presence of proteins was confirmed using specific antibodies.

Detection of adhesion receptors by immunogold labeling and silver enhancement

Before and after incubation with 2 μg/mL G-CSF for 24 hours, at 37°C, EC monolayers were fixed with 4% paraformaldehyde in 0.15M PBS, pH 7.4 (4°C, 10 min), and incubated with monoclonal antibodies against ELAM-1, VCAM-1, and ICAM-1 (1/100 dilution, for 40 min at room temperature) or with unspecific IgG. The adhesion receptors were detected by immunogold labeling and silver enhancement as previously described.[15]

Leukocyte adhesion on EC monolayers

Static conditions

Adhesion studies under static conditions were performed on EC monolayers previously incubated with G-CSF (2 μg/mL) for 24 hours at 37°C. Leukocytes were plated at a concentration of 2×10^3 cells/well and incubated for 3 hours at 37°C in a 5% CO_2 humidified incubator. Cover slips containing EC monolayers were carefully washed with PBS and stained with May-Grunwald and Giemsa to visualize cell adhesion under the light microscope. Images in the light microscope were captured through a video camera and transferred to a personal computer. The number of adherent cells was expressed as the percentage of leukocytes which were adherent to the EC monolayer (% leukocytes per 100 cells).

Flow conditions

Perfusion studies were carried out using a parallel-plate perfusion chamber[16] and blood anticoagulated with low molecular weight heparin (LMWH, 20 U/mL). The simulated perfusion was delivered at a shear rate of 300 s^{-1}, for 10 minutes. After perfusion, the coverslips were processed and analyzed as described above.

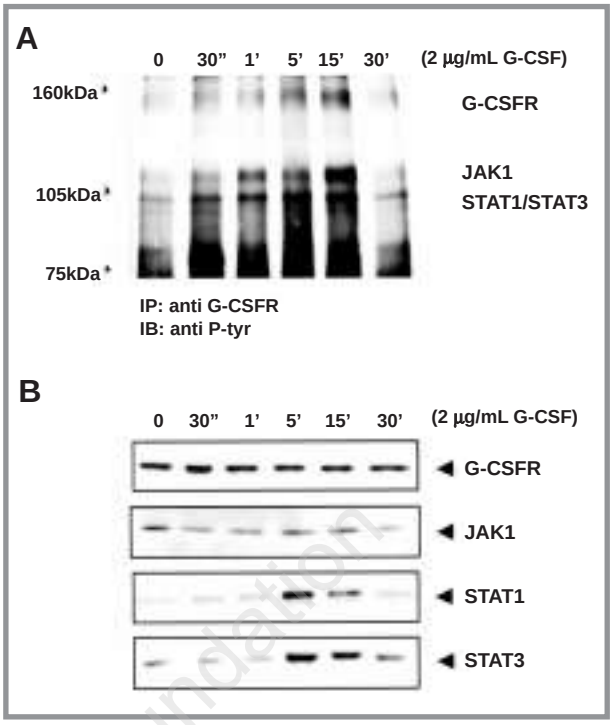

Figure 1. Immunoprecipitation of the G-CSF receptor (G-CSFR) in cell extracts from endothelial cells exposed to 2μg/mL G-CSF for 0, 30 s, 1, 5, 15 and 30 min. Panel A shows the kinetics of tyrosine phosphorylation in immunoprecipitates of the G-CSFR, which resulted in the co-precipitation of JAK-1, STAT-1 and STAT-3. In panel B, presence of the G-CSFR and the associated proteins, JAK-1, STAT-1 and STAT-3, was confirmed. The images are from 1 representative experiment out of 6. IP: immunoprecipitation and IB: immunoblot with an anti-phosphotyrosine antibody conjugated with horseradish peroxidase.

Statistics

Data are expressed as mean±standard error of the mean (SEM). Statistical evaluation of differences between groups of studies was performed using Student's t test for paired data. A $p < 0.05$ was considered statistically significant.

Results

G-CSF triggers the JAK/STAT and the RAS/MAPK pathways in endothelial cells

Tyrosine phosphorylation of proteins in cell lysates was evaluated after the EC had been exposed to different concentrations of G-CSF (0, 0.2, 2, and 20 μg/mL). The maximum intensity of phosphorylation was found when EC were incubated with 2 μg/mL of G-CSF. Using this concentration (2 μg/mL), tyrosine phosphorylation was maximum after 30s of incubation and was not modified along the incubation time (from 30s to 30min).

Immunoprecipitation of the G-CSFR was carried out

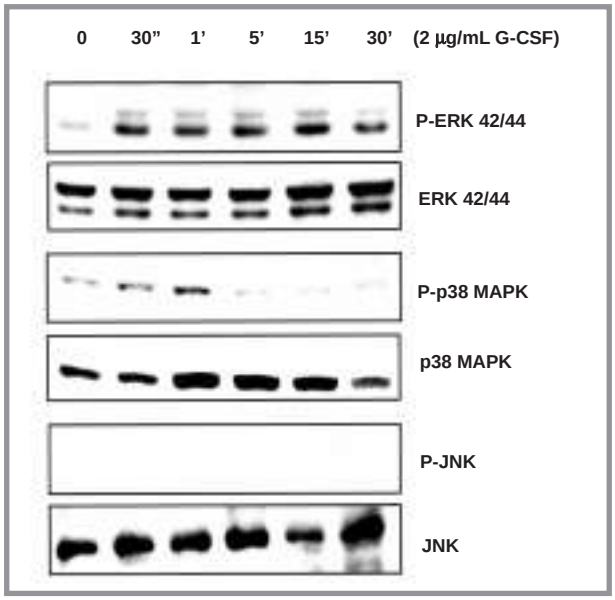

Figure 2. Kinetics of tyrosine phosphorylation of ERK 42/44, p38 MAPK and JNK in endothelial cells exposed to G-CSF. Endothelial cells were exposed to 2 μg/mL G-CSF for 0, 30 s, 1, 5, 15 and 30 min. Blots showing equal amount of proteins ERK 42/44, p38 MAPK and JNK are also included. The images refer to 1 representative experiment out of 6.

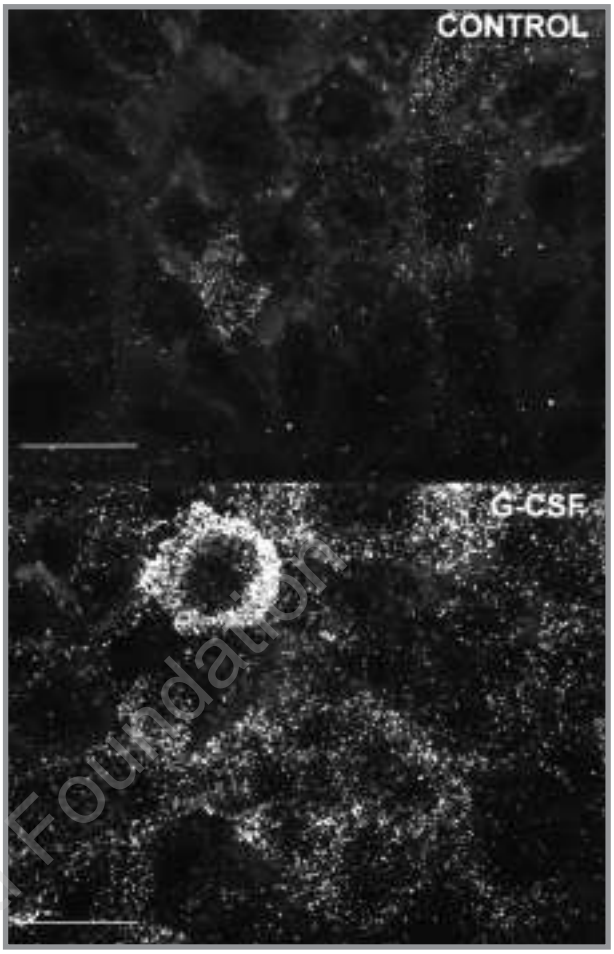

Figure 3. Light micrographs (×400) showing the expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells exposed to G-CSF. Images correspond to untreated cells (CONTROL) and cells treated with 2μg/mL G-CSF for 24 hours (G-CSF). ICAM-1 was detected using immunogold staining and silver enhancement techniques. Images are for 1 representative experiment out of 12. Scale bar corresponds to 10μm.

(n=6) after incubating endothelial cells with 2 μg/mL of G-CSF for 0, 30s, 1, 5, 15, and 30min. Several proteins co-precipitated with the G-CSFR and appeared phosphorylated at tyrosine residues (Figure 1A). When endothelial cells were incubated with 2 μg/mL of G-CSF, a protein of 130kDa, identified as JAK-1, appeared phosphorylated from 30s to 15 min following the same kinetics as the G-CSFR. A band of around 92kDa, which could correspond to STAT-1 and/or STAT-3, was also detected to be tyrosine phosphorylated. The presence of the G-CSFR and the associated proteins, JAK-1, STAT-1 and STAT-3, was confirmed by probing the membranes with specific antibodies (Figure 1B). The G-CSFR receptor and JAK-1, which co-precipitated, were detected to be homogeneously present in all samples corresponding to cell lysates. Association of STAT-1 and STAT-3 to the G-CSFR increased after 5 min of exposure to the cytokine.

The phosphorylation kinetics of the MAPKs, ERK p42/44, p38 MAPK and JNK were individually evaluated using the same experimental design (n=6) (Figure 2). Proteins ERK p42/44 and p38 MAPK appeared to be rapidly phosphorylated once the EC were exposed to 2 μg/mL of G-CSF. While G-CSF induced long-lasting phosphorylation of ERK 42/44 from 30s to 30 min, p38 MAPK was transiently phosphorylated from 30s to 1min. In contrast, although JNK was detected to be present in cell lysates, it was not found to be phosphorylated at any time point. The presence of the ERK p42/44, p38 MAPK and JNK proteins was confirmed to be homogeneous in all the samples evaluated by incubating the immunoblots with specific antibodies (Figure 2).

G-CSF induces the expression of adhesion receptors on the endothelial cell surface

ELAM-1 and VCAM-1 were not found to be expressed in control EC, although ICAM-1 was weakly expressed. Microscopy evaluation of EC monolayers exposed to 2 μg/mL of G-CSF for 24 hours revealed increased presence of the three adhesion receptors, especially at sites of intercellular contacts (Figure 3). Densitometric analysis of the microscopic images confirmed our observations. In monolayers exposed to G-CSF, the intensity of labeling (expressed as pixels per μm^2) for ELAM-1, VCAM-1 and ICAM-1 increased notably from 0.5±0.05 to 2.4±0.1, from 0.7±0.03 to 2.8±0.04, and from 2.8±0.2 to 6.9±0.4,

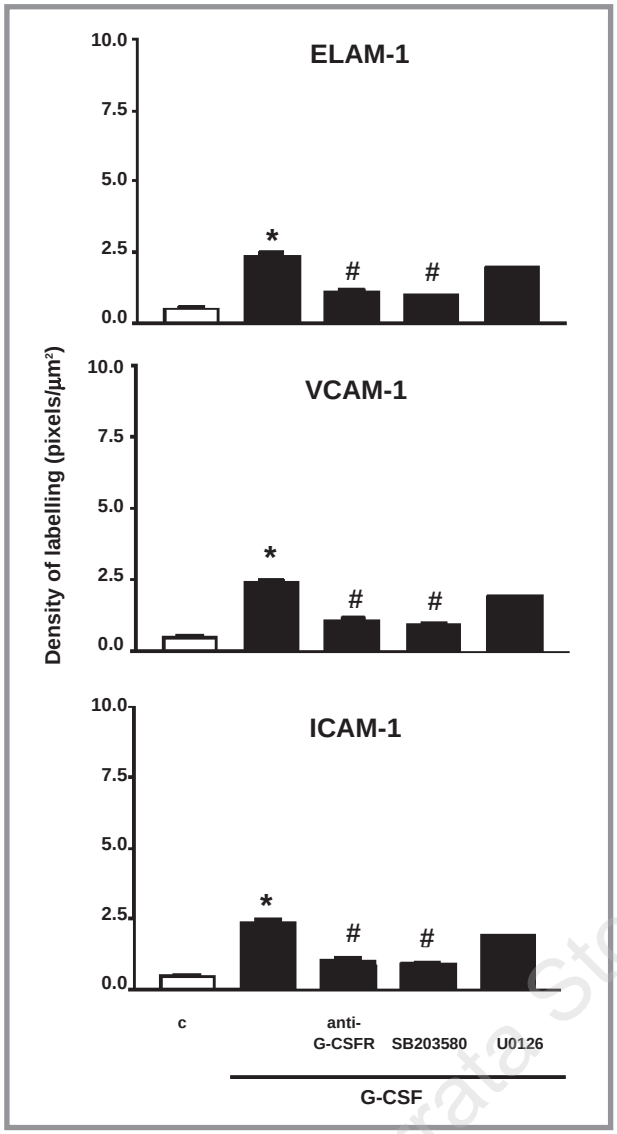

Figure 4. Expression of ELAM-1, VCAM-1 and ICAM-1 on the surface of endothelial cells exposed to G-CSF. Bar diagrams represent data corresponding to levels of adhesion receptors on the surface of cells exposed to 2 μg/mL of G-CSF, for 24 hours. Data are expressed as density of labeling (pixels/μm²) (mean±SEM, n=12) in non-treated EC (C), and EC treated with 2 μg/mL of G-CSF in the absence (G-CSF) and in the presence of an antibody against the G-CSF receptor (Anti-G-CSFR), a specific inhibitor of p38 MAPK (SB203580) and a specific inhibitor of MAPK 42/44 (U0126), *p<0.05 vs control, and #p<0.05 vs 2 μg/mL of G-CSF.

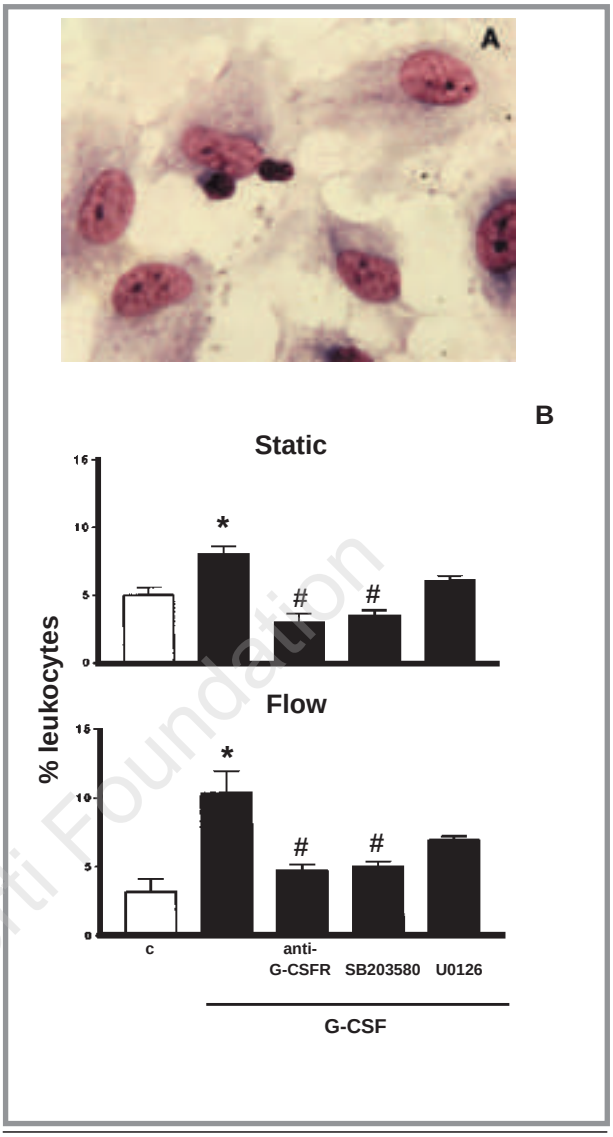

Figure 5. A. Light micrograph showing leukocytes adhered to an endothelial cell monolayer previously exposed to G-CSF, under flow conditions (X800). B. Bar diagrams showing adhesion of leukocytes expressed as the percentage of adhered leukocytes (%leukocytes) under static (Static) and flow (Flow) conditions (mean±SEM, n=6). Substrates are untreated EC (C), and EC treated with 2 μg/mL of G-CSF in the absence (G-CSF) and in the presence of an antibody against the G-CSF receptor (Anti-G-CSFR), a specific inhibitor of p38 MAPK (SB203580) and a specific inhibitor of MAPK 42/44, (U0126) (*p<0.05 vs control, and #p<0.05 vs 2 μg/mL of G-CSF).

respectively (mean±SEM, *p<0.05, n=12) (Figure 4).

Similar experiments were performed in the presence of an antibody against the G-CSFR, SB203580, a specific inhibitor of the p38 MAPK, and U0126, a specific inhibitor of ERK42/44. Under these inhibitory conditions, the density of labeling diminished to basal levels when the G-CSF receptor was blocked or when the p38 MAPK inhibitor of all three adhesion receptors was added. However, when U0126, a specific inhibitor of MAPK42/44, was used, the effect produced by the G-CSF was almost unmodified (Figure 5). Values of density of labeling after EC monolayer exposure to G-CSF in the presence of an antibody to the G-CSFR and in the presence of the inhibitors, SB203580 and U0126, were 1±0.02, 1.1±0.08, and 2±0.01, for ELAM-1 (mean±SEM,

#*p*<0.05, n=12), 0.9±0.1, 1±0.05, and 1.9±0.05, for VCAM-1, and 2.9±0.09, 3.0±0.05, and 5.5±0.3, for ICAM-1.

Effect of G-CSF on leukocyte adhesion to endothelial cells

Adhesion of leukocytes to EC was evaluated under both static and flow conditions. A slight degree of leukocyte attachment was found on control EC monolayers under both conditions, the percentage of leukocyte adhesion being 5.1±0.4% (mean±SEM, n=6) under static conditions and 3.19±0.9% in the flow experiments. When EC were exposed to G-CSF for 24 hours, leukocyte adhesion increased to 8±0.5% under static conditions and to 10.4±1.6% under flow conditions (mean±SEM, #*p*<0.05, n=6) (Figure 5A and B)

The presence of an antibody to the G-CSFR and the inhibitor, SB203580, diminished the effect produced by the cytokine. However, the effect produced by G-CSF was weakly reduced by the ERK42/44 inhibitor, U0126. Under static conditions, values of leukocyte adhesion were 3.1±0.5%, 3.5±0.3%, and 6.2±0.3%, respectively (mean±SEM, #*p*<0.05, n=6). The corresponding values under flow conditions were 4.7±0.5%, 5±0.4%, and 6.5±0.2%, respectively (Figure 5B).

Discussion

Through the present study we show that the interaction of G-CSF to its specific receptor in human endothelial cells resulted in activation of the JAK/STAT signaling pathway. Simultaneously, G-CSF induced long-lasting phosphorylation of MAPK p42/44 and rapid activation of the inflammation-associated protein p38 MAPK. Activation of this latter protein was directly associated with a higher expression of the endothelial adhesion receptors ELAM-1, VCAM-1 and ICAM-1, followed by an increase in leukocyte recruitment. These *in vitro* findings suggest that G-CSF induces a pro-inflammatory effect on endothelial cells.

Endothelial cells may produce G-CSF in response to low numbers of granulocytes.[17] Inflammatory cytokines such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) have been described to induce expression of G-CSF by endothelial cells.[18] Although endothelial cells are not considered hematopoietic cells, they share some features with blood cells through a common progenitor, the hemangioblast.[19] This fact probably explains why they express most of the hematopoietic receptors including the G-CSFR.[8] Several studies have investigated different G-CSFR classes expressed in the neutrophil lineage, but little is known about the G-CSFR on endothelial cells. The fact that we observed the maximum intensity of phosphorylation when cells were exposed to 2 μg/mL of G-CSF fits with the concept that the G-CSF receptor implicated is a low affinity receptor, as has been indicated for the erythropoietin receptor expressed on endothelial cells.[20,21]

Several studies have reported the signaling mechanisms that are activated when G-CSF couples to its receptor in neutrophils;[6,22] but little is known about the effects of G-CSF on endothelial cells. Our experimental data show that G-CSF binding to its receptor activates both JAK/STAT and RAS/MAPK signaling pathways in cultured endothelial cells. Immunoprecipitation of the G-CSFR resulted in the co-precipitation of JAK-1, which appeared rapidly phosphorylated at tyrosine residues. Immunoprecipitates of the G-CSFR contained STAT-1 and STAT-3, which were also tyrosine phosphorylated after incubating endothelial cells with G-CSF. Proteins belonging to the Janus kinase (JAK) family are associated with the cytoplasmic region of most of the cytokine receptors. These proteins are responsible for initiation of the signaling cascade[23] that results in the activation of a group of proteins, known as STAT (signal transducers and activators of transcription). Stimulation of STAT proteins is followed by nuclear translocation to modulate gene expression in their target cells.[24] In addition, JAK proteins phosphorylate other signaling/adaptor proteins, linking JAK signaling to other pathways such as the MAPK pathway. In the present study, we found that the ERK 42/44 and p38 MAPK proteins, which are members of the RAS/MAPK pathway, were also activated in endothelial cells exposed to G-CSF. In contrast, MAPK JNK, which is related to programmed cell death, did not seem to be activated in response to G-CSF.

Protein p38 MAPK is a member of the MAPK family which becomes activated under conditions of stress and inflammation.[25] Under our experimental conditions, p38 MAPK appeared rapidly and transiently phosphorylated in endothelial cells exposed to G-CSF. In relation to this finding, we observed that G-CSF induced the expression of the adhesion receptors ELAM-1 and VCAM-1 and increased the levels of ICAM-1 at the surface of endothelial cells. This effect seemed to be specifically related to the events that follow receptor ligation since it was blocked not only by inhibiting the G-CSFR, but also by a specific inhibitor of p38 MAPK, SB203580. In addition, and as mentioned before, activation of the JAK/STAT and RAS/MAPK signaling pathways is related to gene transcription.[26] Endothelial cells do not possess stored forms of endothelial adhesion receptors[27] and the expression of these receptors is largely dependent on new mRNA synthesis. Accordingly, other authors recently reported significantly elevated plasma levels of soluble endothelial adhesion molecules in healthy donors undergoing progenitor cell mobilization with G-CSF.[10] The increase in observed lev-

els of adhesion receptors could be an indicator of a pro-inflammatory effect of G-CSF. In adition, under our experimental conditions the protein ERK42/44 also appeared to undergo long-lasting phosphorylation after exposure of endothelial cells to G-CSF. Protein ERK42/44 is another member of the MAPK family, although its function is more related to proliferation and differentiation processes.[26] The effects produced by G-CSF on endothelial cells were only weakly inhibited by the addition of U0126, the specific inhibitor of ERK42/44. These results suggest that markers of inflammation detected in EC in response to G-CSF are not related to the ERK42/44 signaling pathway.

Results fron the present study indicate that G-CSF promotes adhesion of leukocytes to EC. Endothelial cells are critical elements in the evolution of all types of inflammation. They participate in the pathologic process through the synthesis and secretion of pro-inflammatory cytokines, including interleukins (IL1, IL6 and IL8), and colony-stimulating factors (G-CSF, M-GCSF, and GM-CSF). They also express a series of cell surface receptors that allow circulating leukocytes to bind selectively to endothelial cells.[28] Experimental studies suggest that G-CSF has a role in the pathogenesis of inflammatory tissue injury. In this regard, G-CSF administration has been shown to exacerbate arthritic symptoms in a mouse model[29] and G-CSF has been found in the synovial fluid of patients with rheumatoid arthritis.[30] Moreover, other authors have reported that G-CSF, besides its ability to drive maturation and mobilization of neutrophils, also has the potential to act on neutrophils promoting their adhesion to endothelial cells.[31]

We hypothesize that the apparent pro-inflammatory properties induced by G-CSF on the endothelium which we observed in our study could be related to an improved homing of hematopoietic progenitor cells into the bone marrow. Endothelial cells, among others, are components of the stromal environment. It is known that during homing processes progenitor cells adhere to the bone marrow through various adhesion receptors, such as VCAM-1 or ICAM-1.[32] Recent research has emphasized the importance of endothelial cells in supporting the expansion of adult bone marrow cells.[9] Future studies should confirm whether or not this hypothesis is correct.

In conclusion, our present data provides the first experimental evidence that G-CSF induces the expression of the adhesion receptors ELAM-1, VCAM-1, and ICAM-1 in endothelial cells, mainly through the activation of p38 MAPK. Moreover, increased expression of adhesion receptors is followed by leukocyte recruitment. Whether or not endothelial cells will be a useful tool for understanding the complicated processes of homing and expansion of hematopoietic progenitor cells in the bone marrow needs further investigation.

BF: conception and design, analysis and interpretation of the data, drafting the article; RM: revising the article and final approval of the version; GE: revising the article and final approval of the version; AM: analysis and interpretation of data; AO: revising the article; MD-R: design of the study and analysis and interpretation of the data, drafting and revising the article and final approval of the version to be published. The authors reported no potential conflicts of interest.

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