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Granulocyte colony-stimulating factor receptors on granulocytes are down-regulated after endotoxin administration to healthy humans

Pascale E. P. Dekkers,1 Nicole P. Juffermans,1 Tessa ten Hove,1 Evert de Jonge,2 Sander J. H. van Deventer,1 and Tom van der Poll1

1 Academic Medical Center, University of Amsterdam, Laboratory of Experimental Internal Medicine, and 2 Department of Intensive Care Medicine, Amsterdam, The Netherlands

Granulocyte colony-stimulating factor (G-CSF) is considered an important mediator of host defense against infection, and recombinant G-CSF is administered to patients with various infections. G-CSF binds to a specific receptor that is expressed on granulocytes and monocytes. To obtain insight about the regulation of the G-CSF receptor after an acute infectious challenge, 8 healthy subjects received an intravenous injection of lipopolysaccharide (LPS; 4 ng/kg), and receptor expression was determined on blood leukocytes by fluorescence-activated cell sorter analysis, both by measurement of saturation binding of recombinant G-CSF and by use of an anti–G-CSF–receptor antibody. LPS induced a transient decrease in granulocyte, but not monocyte, G-CSF–receptor expression. In whole blood in vitro, not only LPS but also gram-positive stimuli and proinflammatory cytokines were capable of down-modulating the G-CSF receptor on granulocytes. Bacterial antigens down-regulate the G-CSF receptor at the surface of granulocytes, which may impair neutrophil functions important for antibacterial host defense.

Granulocyte colony-stimulating factor (G-CSF) is an important regulator of the maturation, proliferation, and function of granulocytes [1, 2]. Major activities of G-CSF on granulocytes include enhancement of chemotaxis, phagocytosis, respiratory burst, and microbicidal activity. As such, G-CSF has been implicated as a pivotal mediator of host defense against infection, and recombinant G-CSF has been advocated as an adjunctive therapy for various infections in neutropenic and nonneutropenic patients [1, 2]. Indeed, administration of recombinant G-CSF has been evaluated in clinical trials with patients with sepsis, pneumonia, diabetic foot infection, candidemia, and wound and burn infections [2].

The biological activity of G-CSF is mediated by specific receptors expressed on the surface of responsive cells. Neutrophils have a high expression of G-CSF receptors (CD114) [3]. Other cell types that express G-CSF receptors include platelets, monocytes, some T- and B-lymphoid cell lines, and several nonhematopoietic cells such as endothelial, placenta, and trophoblastic cells [3]. The monomeric form of the purified G-CSF receptor binds G-CSF with low affinity, whereas the oligomeric form of the receptor shows high affinity binding, which suggests that the high-affinity G-CSF receptor may be formed by a homodimer of the CD114 protein [4].

In vitro experiments have suggested that the expression of G-CSF receptors on granulocytes is influenced by inflammatory stimuli. Indeed, incubation of neutrophils with lipopolysaccharide (LPS), tumor necrosis factor-α (TNF), or C5a resulted in a reduced capacity of granulocytes to bind radiolabeled G-CSF [5, 6]. In accordance with the in vitro experiments, infusion of high doses of recombinant interleukin (IL)-1β was associated with reduced G-CSF binding, by circulating neutrophils in cancer patients in vivo [7]. Knowledge of the regulation of G-CSF receptors on circulating leukocytes during infection is highly limited. Such knowledge is important, in light of the increasing use of G-CSF in infectious diseases. In a first attempt to obtain insight about alterations in G-CSF receptor expression during acute infectious inflammation in vivo, we measured G-CSF receptors on circulating leukocytes before and at various times after an intravenous injection of LPS in healthy humans.

Methods

Human endotoxemia model. Eight men (mean age, 23 years; range, 19–29 years) were admitted to the Clinical Research Unit of the Academic Medical Center of the University of Amsterdam. They received an intravenous injection with Escherichia coli LPS, lot G (United States Pharmacopoeial Convention, Rockville, MD), over 1 min in an antecubital vein at a dose of 4 ng/kg body weight.
All subjects were in good health, as documented by history, physical examination, and hematological and biochemical screening. They did not use any medication or alcohol in the week preceding the study, and they did not smoke or use illicit drugs. Blood for fluorescence-activated cell sorter (FACS) analysis was drawn in heparin-containing vacutainer tubes and immediately put on ice. G-CSF was determined in EDTA plasma by ELISA according to the instructions of the manufacturer (R&D Systems Europe, Abingdon, UK; detection limit, 73.8 pg/mL).

Whole blood stimulation. Whole blood was stimulated as described elsewhere [8, 9]. Heparinized whole blood, diluted 1:2 in sterile RPMI-1640 (Gibco BRL, Grand Island, NY) was stimulated for 1, 2, or 4 h at 37°C with different stimuli in sterile polypropylene tubes (Becton Dickinson, Rutherford, NJ). Each test was performed ≥4 times with blood from different healthy donors. The stimuli used were LPS (from E. coli serotype 0111: B4; Sigma, St. Louis; 10 ng/mL), lipoteichoic acid (LTA; Sigma, 1 g/mL), heat-killed Staphylococcus aureus (HKSA, reference strain 14459B from the National Institute of Public Health and the Environment, Bilt- hoven, the Netherlands; 10^7 cfu/mL), staphylococcal enterotoxin B (SEB; Sigma, 1 g/mL), recombinant human interferon-γ (IFN-γ; R&D Systems, 10 ng/mL), and recombinant human TNF (kindly provided by Knoll, Ludwigshafen, Germany; 10 ng/mL). After incubation, blood was immediately put on ice and processed for FACS analysis as described below.

FACS analysis. For FACS analysis, erythrocytes were lysed with ice-cold isotonic NH_4Cl solution (155 mmol/l NH_4Cl, 10 mmol/l KHCO_3, 0.1 mmol/l EDTA, pH 7.4) for 10 min. Cells were centrifuged at 600 g for 5 min at 4°C. The remaining cells were brought to a concentration of 4×10^6 cells/mL in FACS buffer (PBS supplemented with 0.5% bovine serum albumin, 0.01% NaN_3, and 100 mM EDTA). Expression of cell-associated G-CSF receptor was determined by two methods. Saturation binding of G-CSF by white blood cells was determined with phycoerythrin (PE)-labeled human G-CSF (G-CSF-PE; Fluorokine; R&D Systems). The specificity of the G-CSF-PE reaction was evaluated in two ways (i.e., a blocking anti-G-CSF-receptor antibody reduced the number of neutrophils that stained with G-CSF-PE to <7%, and a 10-fold molar excess of unlabeled G-CSF reduced the number of cells that stained with G-CSF-PE by 98% [information provided by the manufacturer]). In addition, G-CSF-receptor expression was evaluated using a mouse anti-human G-CSF-receptor antibody (clone LMM775; Instruchemie, Hilversum, the Netherlands). Both reagents were used in concentrations recommended by the manufacturer. To correct for nonspecific staining, an appropriate control antibody (murine IgG_1; Becton Dickinson) was used in the experiments in which the anti-G-CSF-receptor antibody was used. For each test, ≥10^6 cells were counted. Mean cell fluorescence (MCF) at >570 nm of forward and side-angle scatter-gated monocytes and granulocytes was assessed. Data are presented as the difference between mean channel fluorescence (MCF) and nonspecific MCF (mean ± SE; n = 8).

Results

Endotoxemia in healthy subjects. At baseline, G-CSF receptor was detectable at the surface of peripheral blood monocytes and granulocytes, using either saturation binding of recombinant G-CSF or binding of an anti-G-CSF-receptor antibody as the method of detection. LPS administration was associated with a decrease in the surface expression of G-CSF receptor on granulocytes reaching nadirs after 2–4 h (MCF for binding of anti-G-CSF-receptor antibody: 0 h, 208.6 ± 60.3; 2 h, 48.6 ± 13.4, P = .01; MCF for binding of recombinant G-CSF: 0 h, 92.0 ± 29.3; 4 h, 7.9 ± 3.3; P = .001; figure 1). In
Figure 2. Effect of lipopolysaccharide (LPS) and other inflammatory stimuli on granulocyte colony-stimulating factor (G-CSF)–receptor on granulocytes in whole blood in vitro. Upper panel, LPS (10 ng/mL), which causes a time-dependent down-regulation of the granulocyte G-CSF receptor. Lower panel, Gram-positive stimuli and cytokines, which induce down-regulation of the granulocyte G-CSF receptor. Whole blood was incubated for 4 h in the presence or absence of lipoteichoic acid (LTA, 1 μg/mL), heat-killed Staphylococcus aureus (HKSA, 10^7 cfu/mL), staphylococcal enterotoxin B (SEB, 1 μg/mL), recombinant human interleukin-6 (10 ng/mL), recombinant human interferon (IFN-γ) (10 ng/mL), and recombinant human tumor necrosis factor (TNF; 10 ng/mL). Bars represent the difference between specific mean channel fluorescence (MCF) and nonspecific MCF of 4 different donors (mean ± SE).

Discussion

Intravenous injection of low-dose LPS represents a well-accepted, reproducible model to study the early responses to an acute bacterial challenge in humans [10]. In the present study, we used this model to assess alterations in the expression of G-CSF receptors on circulating leukocytes induced by LPS in vivo. LPS was found to down-modulate G-CSF receptors on granulocytes selectively while not influencing the expression of G-CSF receptors on monocytes. Additional in vitro experiments with whole blood revealed that not only LPS, but also gram-positive antigens, down-regulate G-CSF receptors. Recent studies have indicated that functional G-CSF receptors play an important role in normal neutrophil activation induced not only by G-CSF itself but also by various chemoattractants, including IL-8 [11]. Together, these data suggest that bacterial antigens may reduce the responsiveness of granulocytes to G-CSF and thereby exert a more general inhibitory effect on neutrophil function.

The expression of G-CSF receptors was evaluated with two methods. G-CSF binds to cells with high affinity only when two G-CSF receptors have formed a homodimer [3, 4]. We therefore considered it to be of interest to study both saturation binding of recombinant G-CSF–PE, providing direct information on the capacity of cells to interact with G-CSF, and binding of an anti–G-CSF–receptor antibody, providing information on the extent of expression of the number of individual G-CSF receptors. Both methods yielded similar results, supporting the main finding of this study—that is, that acute inflammation results in a reduced capacity of granulocytes to interact with G-CSF. It is remarkable that monocytes, which expressed G-CSF receptors on their surface to a much lesser extent than granulocytes, did not react to LPS with a down-modulation of G-CSF receptors. In a previous study, TNF, but not IL-1 or IFN-γ, was found to attenuate the binding of radiolabeled G-CSF to murine peritoneal exudate macrophages [12], which suggests that more differentiated mononuclear cells may respond to an inflammatory stimulus in a different way.

In contrast, G-CSF–receptor expression did not change on monocytes after LPS injection (figure 1).

Plasma concentrations of G-CSF increased markedly after LPS injection, peaking after 4 h (1819 ± 356 pg/mL, P < .001) and returning to baseline after 12 h.

Whole blood stimulation in vitro. Incubation of whole blood with LPS caused a time-dependent decrease of G-CSF–receptor expression on granulocytes, compared with unstimulated blood. LPS was found to decrease granulocyte G-CSF–receptor expression after only 1 h of incubation, when either method of G-CSF–receptor detection was used. G-CSF–receptor expression reached a nadir after 4 h of incubation (figure 2, upper panel). In accordance with the in vivo experiments, monocyte G-CSF receptor were not influenced by LPS (data not shown).

Having established that the LPS effects in vivo could be reproduced in whole blood in vitro, we used this system to assess whether other bacterial stimuli could influence G-CSF–receptor expression. To do so, whole blood was incubated for 4 h in the presence or absence of stimuli derived from gram-positive bacteria—that is, HKSA, LTA (a cell wall component of S. aureus), and SEB (a superantigen produced by S. aureus). All of these gram-positive stimuli decreased G-CSF–receptor expression on granulocytes (figure 2, lower panel). In addition, the proinflammatory cytokines TNF and IFN-γ also down-modulated G-CSF–receptor expression on this cell type (figure 2, lower panel).
The functional consequences of alterations in G-CSF–receptor expression on monocytes/macrophages are unclear, inasmuch as G-CSF does not seem to have important biological effects on these cells [1, 2].

The G-CSF receptor has structural homology with gp130, a transmembrane molecule that serves as a common signal-transducing element for the IL-6 family of cytokines [3]. We recently reported that intravenous injection of LPS does not influence the expression of gp130 on monocytes or granulocytes [9], which indicates that the expression of the G-CSF receptor and gp130 is regulated differentially. In contrast, the IL-6 receptor, the ligand-binding part of the IL-6 receptor complex, was down-modulated on both monocytes and granulocytes after in vivo exposure of healthy humans to LPS [9]. Similarly, other cytokine receptors on monocytes and granulocytes also become down-modulated after LPS administration, including the receptors for TNF and IL-1 [8]. Likewise, IL-8 receptors demonstrate a reduced expression on granulocytes after injection of LPS into humans (authors’ unpublished data). It is interesting that yet another receptor important for leukocyte chemotaxis, the receptor for urokinase-type plasminogen activator (uPAR, CD87), was found to be up-regulated on monocytes, but not on granulocytes, during human endotoxemia [13]. These data indicate that surface receptors relevant for antibacterial host defense on monocytes and granulocytes respond in a complex way and sometimes in opposite directions, at the first interaction with a bacterial stimulus.

The data obtained in healthy humans exposed to LPS in vivo apply only to granulocytes that remained in the circulation. It therefore cannot be excluded that G-CSF receptors on granulocytes that adhere to the vascular endothelium shortly after LPS challenge behave differently from G-CSF receptors on granulocytes drawn from peripheral blood.

LPS injection was associated with an increase in the plasma concentrations of G-CSF. This finding confirms an earlier LPS challenge study in healthy humans [14] and is in line with elevated circulating levels of G-CSF in patients with acute infections [15].

G-CSF is often used as prophylaxis and treatment of patients with disseminated Candida infections [1, 2]. In our study, the effect of Candida antigens on G-CSF receptor expression was not investigated. Additional studies are warranted to establish whether the down-regulating effect of bacterial antigens on G-CSF receptors also apply to Candida antigens.

G-CSF has gained widespread interest because of its potential beneficial effects during infections. Our present findings suggest that granulocytes, which comprise the main target cells for G-CSF, have a reduced capacity to interact with G-CSF shortly after they are exposed to bacterial antigens. Bacterial infection may lead to impaired neutrophil functions secondary to a reduction in G-CSF receptors, and granulocytes from patients with severe infections may be less responsive to exogenously administered G-CSF.

References