Endotoxin Down-Modulates Granulocyte Colony-Stimulating Factor Receptor (CD114) on Human Neutrophils

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During infection, the development of nonresponsiveness to granulocyte colony-stimulating factor (G-CSF) may be influenced by the down-modulation of G-CSF receptor (G-CSFR) by cytokines. This down-modulation was studied during experimental human endotoxemia. Healthy volunteers received either 2 ng/kg endotoxin (lipopolysaccharide [LPS], n = 20) or placebo (n = 10) in a randomized, controlled trial. Endotoxin infusion increased the mean fluorescence intensity of the neutrophil activation marker CD11b >300% after 1 h (P < .001 vs. placebo). LPS infusion down-modulated G-CSFR expression in as early as 60 min (−17%; P = .001 vs. placebo). Down-modulation was almost maximal at 90 min and persisted for 6 h (−50% from baseline; P < .0001 vs. placebo). Plasma levels of G-CSF started to increase only after G-CSFR down-modulation had occurred and peaked 37-fold above baseline at 4 h (P < .0001 vs. placebo). In conclusion, LPS down-modulates G-CSFR expression in humans, which may render neutrophils less responsive to the effects of G-CSF and, thereby, compromise host defense mechanisms.

Granulocyte colony-stimulating factor (G-CSF) is crucial for proliferation of neutrophils [1], delays apoptosis of neutrophils [2], and enhances neutrophil functions, such as oxidative burst [3] and phagocytic and bactericidal activity [4].

Endogenous plasma levels of G-CSF are normally in the low picomolar range [1–5]. In patients undergoing a successful response to infection, G-CSF levels are high in the acute phase and decrease consecutively [5]. G-CSF is removed by binding to its receptor (G-CSFR, CD114) and in reaction to increased neutrophil counts [1], which contribute substantially to clearance of G-CSF.

Endogenous G-CSF concentrations are initially very high in bacteremic patients [5, 6]. This could be explained in part by decreased clearance due to so-called trans down-modulation of G-CSFR surface expression on neutrophils by mediators such as lipopolysaccharide (LPS) or cytokines (i.e., down-modulation by a stimulus other than G-CSF). This heterologous G-CSFR down-modulation has been observed in vitro [7], and, if it occurs in vivo, it could play an important role in the development of nonresponsiveness to G-CSF and the occurrence of relative or absolute neutropenia in sepsis. It could prevent further recruitment of neutrophils from the bone marrow into the circulation and, thereby, decrease host defense mechanisms.

We studied trans down-modulation of G-CSFR during experimental human endotoxemia [8]. We characterized the time course of G-CSFR expression and G-CSF plasma levels, in healthy, LPS-challenged volunteers, and their relation to leukocyte activation, as measured by expression of the β2 integrin CD11b.

Methods

Study design. The study design was 2:1 randomized (LPS: placebo), double blind, placebo controlled, and in parallel groups.

Study subjects. Healthy male volunteers were invited to participate in this trial. All subjects were 19–35 years of age with a body mass index between the 15th and the 85th percentile. Determination of health status included medical history, physical examination, laboratory parameters, and virologic and standard drug screening. In addition, study subjects were tested for hereditary thrombophilia (i.e., factor V Leiden, protein C, and protein S deficiency), to minimize potential risks imposed by endotoxin-induced coagulation activation. Exclusion criteria were regular or recent intake of medication, including nonprescription medication, and relevant abnormal findings in medical history or laboratory parameters.

Study protocol. The experimental procedures of our endotoxin
infusion studies have been described in detail elsewhere [9]. In brief, volunteers were admitted to the study ward at 8:00 AM after an overnight fast, because the response to endotoxin varies with the time of day [10]. Throughout the study period, subjects were confined to bed rest and kept fasting for 8.5 h after LPS infusion. A 5% glucose infusion (Leopold Pharma, Vienna) was started at 8:30 AM and continued over 8.5 h at 3 mL/kg/h, to maintain adequate blood glucose levels and urinary output. Thirty minutes after the start of glucose, 20 subjects received an intravenous bolus of 2 ng/kg LPS (National Reference Endotoxin, Escherichia coli; USP Convention, Rockville, MD), 10 other subjects served as a control group, receiving normal saline solution instead of LPS.

Sampling and analysis. Analysts were blinded with regard to group allocation. Sampling times were selected on the basis of the kinetics of G-CSFR down-regulation in pilot subjects challenged with LPS and on the kinetics of homologous G-CSFR down-regulation (i.e., down-modulation of G-CSFR by infusion of G-CSF in humans; unpublished data). Blood samples were obtained by venipuncture and collected into EDTA-anticoagulated Vacutainer tubes (Becton Dickinson, Vienna) before LPS infusion; thereafter, blood was obtained at times indicated in the figures (except samples for leukocyte counts, which were obtained from an indwelling venous line on the contralateral arm, into which LPS had been administered).

Plasma samples were processed immediately by centrifugation at 2000 g at 4°C for 15 min and stored at −80°C before analysis. G-CSF plasma levels were analyzed with a high-sensitivity EIA (R&D Systems, Oxon, UK) [11], and samples from individual subjects were run in the same assay. G-CSFR expression and CD11b expression were quantified by flow cytometry (FACSCalibur; Becton Dickinson). The anti G-CSFR antibody (Serotech, Kidlington, UK) [11] and the anti-CD11b antibody were phycoerythrin labeled (Becton Dickinson). Results are presented as mean fluorescence intensity (MFI). Neutrophil counts were obtained with a cell counter (Sysmex, Kyoto, Japan).

Data analysis. Data are expressed as the mean and the 95% confidence intervals for description in the text. Nonparametric statistics were applied. All statistical comparisons within groups were done with the Friedman analysis of variance and the Wilcoxon signed ranks test for post hoc comparisons. The Mann-Whitney U test was used to test changes in end points between groups for statistical significance. *P* < .05 (2-tailed) was considered significant.

Results

Endotoxin infusion rapidly induced neutrophil activation within 1 h, as measured by surface expression of CD11b, an adhesion molecule and marker of leukocyte degranulation (figure 1). Basal MFI of CD11b ranged from 49 to 1114, with no differences between groups. In line with the observation that LPS increases CD11b expression at 3 h [12], we have now characterized in more detail the time course of CD11b (figure 1): LPS infusion had already enhanced CD11b expression ≥300% after 60 min (*P* < .0005 vs. baseline; *P* = .0002 vs. placebo), and the increase in CD11b expression persisted for at least 6 h.

Baseline MFI of G-CSFR expression averaged 56 (range, 40–67; *P* > .05 between groups). LPS infusion down-modulated G-CSFR expression as early as 60 min (−17%; *P* < .001 vs. baseline; *P* = .001 between groups; figure 1). Down-modulation was almost maximal at 90 min and persisted for 6 h (−50% from baseline), whereas no significant decrease was observed in the placebo group.

Baseline G-CSF plasma levels averaged 19 pg/mL (range, 7–60 pg/mL; *P* > .05 between groups). Of note and in agreement with data elsewhere [12], G-CSF plasma levels did not change during the first hour after LPS infusion (figure 1), but they did increase after 2 h. LPS increased G-CSF plasma levels 37-fold at 4 h, whereas placebo had no effect (*P* > .05). As expected [9], neutrophil counts dropped sharply 60 min after LPS infusion and increased significantly above baseline from 2 h to at least 7 h (figure 2).

Baseline activation of neutrophils (i.e., CD11b expression) cor-
infusion induces heterologous transversely correlated, at baseline. 

\[ P = 0.0027 \]

related negatively with expression of G-CSFR \((r = -0.53, P = 0.0027)\). G-CSFR expression and G-CSF levels were inversely correlated \((r = -0.38, P = 0.044)\) at baseline.

Discussion

The major aim of this study was to examine whether LPS infusion induces heterologous trans down-modulation of G-CSFR on neutrophils in vivo, as was observed in vitro [7]. Indeed, our results demonstrate that LPS down-modulates CD114 expression on the neutrophil surface within 60 min after intravenous LPS (figure 1). This decrease in G-CSFR occurred before any increase was seen in plasma levels of G-CSF (figure 1), which cause homologous G-CSFR down-modulation (unpublished data). Concomitant with the G-CSFR down-modulation, we observed massive activation of neutrophils, as evidenced by the increase in CD11b expression (figure 1). Our results thus confirm in vitro observations elsewhere that LPS trans down-modulates G-CSFR either directly [7] or by a mediator, such as tumor necrosis factor [13].

Down-modulation of G-CSFR by endotoxin is of clinical interest for several reasons. First, decreased expression of G-CSFR is expected to enhance G-CSF levels by decreasing clearance. Together with increased G-CSF release during inflammation, this may partly contribute to the high G-CSF concentrations seen in bacteremic patients [5, 6]. Second, G-CSF concentrations decrease with recovery in survivors of sepsis but remain increased in those who do not survive [14]. We postulate that this difference is primarily due to a difference in G-CSF plasma clearance because of a lack of availability of G-CSFR. Thus, heterologous G-CSFR down-modulation could play an important role in the development of nonresponsiveness to G-CSF and the occurrence of neutropenia in sepsis: it could prevent further recruitment of neutrophils from the bone marrow into the circulation and thereby decrease host defense mechanisms. As far as neutrophil counts are concerned, it appears that the early LPS-induced neutrophilia observed at 2 h was not due to G-CSF, because infusion of exogenous G-CSF increases neutrophil counts only after a time lag of 2 h (unpublished data).

Last, the observed correlations, although not proof of a cause-effect relationship, may reflect the following biologic mechanisms: since G-CSF levels and G-CSFR expression were inversely correlated, it appears that the G-CSFR/ligand pair exert a negative feedback on each other. The highly significant negative correlation between the expression of CD11b and G-CSFR at baseline indicates that neutrophil activation and G-CSFR expression are tightly coregulated even in healthy subjects.

The time course of CD11b expression was examined in more detail in this study because CD11b can function as a receptor for a large number of molecules, including ICAM-1, LPS, iC3b, high–molecular-weight kininogen, fibrinogen, and coagulation factor X [15]. As a consequence, it may be hypothesized that early up-regulation of this adhesion molecule plays an essential role in the very early phase of adhesion to and phagocytosis of bacteria. Further, CD11b expression may contribute to an alternative pathway of LPS-induced coagulation [15]. Thus, CD11b could represent an interesting therapeutic target in sepsis.

In conclusion, LPS trans down-modulates G-CSFR expression in humans, which may render neutrophils less responsive to the effects of G-CSF and thereby compromise host defense mechanisms.

References

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