Endotoxin down-regulates monocyte and granulocyte interleukin-6 receptors without influencing gp130 expression in humans


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Endotoxin Down-Regulates Monocyte and Granulocyte Interleukin-6 Receptors without Influencing gp130 Expression in Humans

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Interleukin (IL)-6 is important for host defense against various pathogens. The IL-6 receptor (IL-6R) complex consists of a ligand-binding component (IL-6R) and a signal-transducing component (gp130). In a study designed to obtain insight into the regulation of this receptor complex during inflammation, 8 healthy subjects received an intravenous injection of lipopolysaccharide (LPS; 4 ng/kg), and receptor expression was determined on blood leukocytes by use of fluorescence-activated cell cytometry. LPS induced a transient decrease in monocyte and granulocyte IL-6R expression but did not influence gp130. The plasma concentrations of soluble IL-6R and soluble gp130 did not change after LPS administration. Expression of the receptor for leukemia inhibitory factor, a member of the IL-6R family, remained unaltered after LPS injection. In whole blood in vitro, LPS and gram-positive stimuli and proinflammatory cytokines were capable of down-modulating the IL-6R. Monocytes and granulocytes may down-regulate IL-6R at their surface upon their first interaction with bacterial antigens.

Serum and plasma concentrations of interleukin (IL)-6, a glycosylated cytokine, increase in a variety of infectious and noninfectious conditions, including endotoxemia and sepsis [1]. IL-6 can be detected most consistently in the circulation of patients with sepsis, and the extent of IL-6 release in these patients carries a strong predictive value for lethality [2, 3].

IL-6 belongs to a family of cytokines that also comprises IL-11, ciliary neurotrophic factor, cardiotrophin-1, leukemia inhibitory factor (LIF), and oncostatin M [4]. All members of the IL-6 cytokine family share the transmembrane molecule gp130 as a common signal-transducing element on responsive cells. The functional IL-6 receptor (IL-6R) complex consists of IL-6R (CD126) and gp130. IL-6R serves as the IL-6-binding component of the receptor complex, which by itself does not induce signal transduction. Instead, the IL-6/IL-6R complex induces the formation of a gp130/gp130 homodimer, which results in a cellular response initiated by activation of JAK kinases [4]. Stimulation of the IL-6R complex results in a variety of biologic effects, including enhancement of acute-phase protein synthesis in the liver, increased megakaryopoiesis, and stimulation of immunoglobulin production by B cells and of a number of systemic proinflammatory (e.g., stimulation of coagulation) and anti-inflammatory (e.g., inhibition of tumor necrosis factor [TNF] production) effects [1, 4]. Soluble forms of both IL-6R and gp130, representing the extracellular domains of the respective surface proteins, can be detected in the circulation of healthy persons [5, 6]. Soluble IL-6R can exert agonistic activity by enhancing the biologic effects of IL-6 [7–9], whereas soluble gp130 functions as an IL-6 antagonist [6, 10].

A number of stimuli induce shedding of soluble IL-6R from the cell surface, including bacterial pore-forming toxins, FMLP, and C-reactive protein [11–13]. In accordance, elevated levels of circulating soluble IL-6R are found in several inflammatory diseases, such as rheumatoid arthritis, multiple myeloma, human immunodeficiency virus (HIV) infection, and malaria [5, 14–16]. In patients with sepsis, however, circulating soluble IL-6R concentrations are decreased [17]. Soluble gp130 levels are not higher in patients with severe malaria than in healthy controls [16].

The human endotoxemia model is widely used as a model of systemic inflammation with relevance for early pathogenetic mechanisms operative during gram-negative infection [18]. Intravenous injection of endotoxin (lipopolysaccharide [LPS]) into normal subjects results in inflammatory responses that qualitatively mimic changes found in patients with sepsis. In the present study, we sought to determine the in vivo effect of LPS on the expression of IL-6R and gp130 on circulating monocytes and granulocytes. Knowledge of the expression of the IL-6R complex may have relevance for possible alterations in IL-6R sensitivity of these cell types. In addition, we assessed changes in the cell surface expression of the receptor for LIF (LIF-R), a member of the IL-6R family, and in the plasma concentrations of soluble IL-6R and gp130. Finally, we eval-
uited the effect of gram-positive stimuli and proinflammatory cytokines on expression of the IL-6R complex in whole blood in vitro.

Subjects and Methods

Human endotoxemia model. Eight men (mean age, 23 years; range, 19–29) were admitted to the Clinical Research Unit of Academic Medical Center in Amsterdam. Each received an intravenous injection of Escherichia coli LPS (lot G; US Pharmacopeial 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 hematologic and biochemical screening. Blood for fluorescence-activated cell cytometry (FACS) analysis was obtained directly before LPS administration (t = 0 h) and at 1, 2, 4, 6, and 24 h thereafter. These blood samples were drawn in heparin-containing vacutainer tubes and immediately put on ice. Blood for ELISA was obtained directly before LPS administration (t = 0 h) and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. This blood was drawn in K$_2$-EDTA–containing tubes and centrifuged at 2000 g for 20 min at 4°C. The plasma was stored at −20°C until the assay was done.

Assays. The following ELISAs (with detection limits) were used according to manufacturers’ instructions: IL-6 (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service [CLB], Amsterdam; 2.2 pg/mL), soluble IL-6R (CLB; 2.04 ng/mL), soluble gp130 (R&D Systems Europe, Abingdon, UK; 20 ng/mL), and LIF (R&D Systems; 23.4 pg/mL).

Whole blood stimulation. Whole blood was stimulated as described elsewhere [19, 20]. Blood was collected aseptically from healthy subjects by use of a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson, Rutherford, NJ). For anticoagulation, we used sterile heparin (LEO Pharmaceutical, Weesp, The Netherlands; final concentration, 10 U/mL blood). Whole blood diluted 1:2 in sterile RPMI 1640 (Gibco BRL Life Technologies, Grand Island, NY) was stimulated for 30 min and 1, 2, or 4 h at 37°C with different stimuli in sterile polypropylene tubes (Becton Dickinson). For these experiments, polypropylene tubes were filled with 1 mL of RPMI containing the appropriate concentrations of the stimuli, after which we added 1 mL of heparinized blood. Following gentle mixing, tubes were placed in the incubator. Each test was done at least 4 times with blood from different healthy donors. The stimuli used were LPS from E. coli O111:B4, 10 ng/mL (Sigma, St. Louis); lipoteichoic acid (LTA) from Staphylococcus aureus, 1 µg/mL (Sigma); heat-killed S. aureus (HKSA), 10$^5$ cfu/mL; staphylococcal enterotoxin B (SEB), 1 µg/mL (Sigma); recombinant human IL-6, 10 ng/mL (CLB); recombinant human interferon (IFN)-γ, 10 ng/mL (R&D Systems); and recombinant human TNF-α, 10 ng/mL (Knoll, Ludwigshafen, Germany). 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$_4$Cl solution (155 mM/L NH$_4$Cl, 10 mM/L KHCO$_3$, and 0.1 mM/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$^5$ 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 IL-6R was determined by use of a mouse anti-human IL-6R monoclonal antibody (clone M91; Immunotech, Marseille, France) in concentrations recommended by the manufacturer. Expression of cell-associated gp130 was determined by use of a mouse anti-human gp130 monoclonal antibody (clone B-R3; Biosource Europe, Nivelles, Belgium) according to the manufacturer’s instructions. To correct for aspecific staining, we used an appropriate control antibody (murine IgG1; Becton Dickinson). Saturation binding of LIF by white blood cells was determined with biotinylated recombinant human LIF (Fluorokine; R&D Systems). For each test, 10$^5$ 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 MCF intensities of specifically and nonspecifically stained cells.

Statistical analysis. Statistical data are presented as mean ± SE. Changes in time were analyzed by 1-way analysis of variance. P < .05 (2 sided) was considered significant.

Results

Endotoxemia in healthy subjects. Injection of LPS induced a decrease in peripheral blood monocytes (P < .05; table 1). Granulocyte counts initially decreased and then increased after 2 h (P < .05; table 1). At baseline, IL-6R, gp130, and LIF-R were detectable at the surface of peripheral blood monocytes and granulocytes (figure 1). LPS administration was associated with a decrease in the surface expression of IL-6R on both cell types, which reached nadirs after 2–6 h (monocytes: 0 h, 115.8 ± 16.5; 2 h, 37.0 ± 8.4, P < .001; granulocytes: 0 h, 137.0 ± 22.3; 6 h, 34.3 ± 5.5, P = .001; figure 2, upper panels). In contrast, gp130 expression did not change on monocytes or granulocytes after LPS injection (figure 3, upper panels). Furthermore, for both cell types, LIF-R expression remained unchanged during endotoxemia (data not shown).

Soluble IL-6R and soluble gp130 were detectable in plasma of all 8 volunteers at baseline (398.8 ± 35.8 and 236.6 ± 22.3 ng/mL, respectively). LPS administration did not influence the concentrations of these soluble receptors (figure 2, figure 3, lower panels). At baseline, neither IL-6 nor LIF was detectable in plasma. Plasma IL-6 concentrations increased strongly after LPS injection, peaking after 3 h (5.99 ± 1.14 ng/mL, P < .05).

Table 1. Effect of intravenous lipopolysaccharide (LPS) on numbers of circulating monocytes and granulocytes.

<table>
<thead>
<tr>
<th>Time, h</th>
<th>Monocytes$^a$</th>
<th>Granulocytes$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.45 ± 0.04</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>1</td>
<td>0.04 ± 0.02</td>
<td>0.7 ± 0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.05 ± 0.03</td>
<td>3.0 ± 0.5</td>
</tr>
<tr>
<td>4</td>
<td>0.08 ± 0.03</td>
<td>7.0 ± 0.7</td>
</tr>
<tr>
<td>6</td>
<td>0.20 ± 0.03</td>
<td>10.2 ± 0.8</td>
</tr>
<tr>
<td>24</td>
<td>0.65 ± 0.04</td>
<td>8.6 ± 0.8</td>
</tr>
</tbody>
</table>

NOTE. LPS (4 ng/kg) was given as intravenous injection at t = 0 h. Data are mean ± SE cells × 10$^9$/L of 8 healthy volunteers.

$^a$ P < .05 for changes in time by analysis of variance.
whereas LIF remained undetectable in plasma throughout the study.

Whole blood stimulation in vitro. Incubation of whole blood with LPS caused a time-dependent decrease of monocyte and granulocyte IL-6R expression in comparison with unstimulated blood. LPS-induced down-modulation of IL-6R expression became apparent after 30 min to 1 h of incubation (figure 4). The expression of gp130 on monocytes and granulocytes decreased spontaneously during the 4-h incubation without stimulus. In accordance with the in vivo experiments, the influence of LPS on monocyte and granulocyte gp130 did not differ from its influence on control incubations (figure 5). Furthermore, LIF-R expression remained unchanged (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 inflammatory stimuli could influence IL-6R expression. For this, whole blood was incubated for 4 h in the presence or absence of stimuli derived from the gram-positive bacteria HKSA, LTA (a cell wall component of S. aureus), and SEB (a superantigen produced by S. aureus). All of these gram-positive stimuli decreased IL-6R expression on monocytes and granulocytes (figure 6, upper panel). In addition, TNF, IFN-γ, and IL-6 also down-modulated IL-6R expression on these cell types (figure 6, lower panel).

Discussion

IL-6 acts on cells via an interaction with a functional receptor complex that is composed of the ligand-binding IL-6R and the
Figure 3. Monocyte and granulocyte gp130 and plasma soluble gp130 after intravenous injection of lipopolysaccharide (lot G, 4 ng/kg) into 8 healthy subjects. Cellular gp130 expression was determined by flow cytometry (see Subjects and Methods section). Results are expressed as difference between specific mean channel fluorescence (MCF) and nonspecific MCF. NS, not significant by 1-mean way analysis of variance (vs. time).

Figure 4. Lipopolysaccharide (LPS) causes time-dependent down-regulation of monocyte and granulocyte interleukin-6 receptor (IL-6R) in whole blood in vitro. Whole blood was incubated for indicated periods in presence or absence of LPS (10 ng/mL). Bars show difference between specific mean channel fluorescence (MCF) and nonspecific MCF of 4 different donors (mean ± SE).

signal-transducing gp130. Although IL-6 has been implicated in host defense against a variety of pathogens, including *Listeria monocytogenes*, *E. coli*, *Streptococcus pneumoniae*, *Candida albicans*, and *Mycobacterium tuberculosis* [21–25], relatively little is known about the regulation of its receptor complex in inflammatory conditions in vivo. Here we demonstrate that in humans LPS induces a down-regulation of IL-6R on circulating monocytes and granulocytes while not influencing the expression of gp130. This effect could be reproduced in whole blood in vitro, in which gram-positive stimuli and the proinflammatory cytokines TNF and IFN-γ were also capable of down-regulating cellular IL-6R. Although the role of TNF and IFN-γ in the LPS effect on cellular IL-6R remains to be established, it seems unlikely that cytokines or other inflammatory mediators contribute to LPS-induced down-modulation of the cell surface of IL-6R in vitro, considering the rapidity of this response (within 30 min). Further studies are warranted to assess the involvement of cytokines in this LPS effect in vivo. Plasma concentrations of soluble IL-6R and gp130 remained unchanged after LPS injection. These data suggest that monocytes and granulocytes may become less responsive to IL-6 when exposed to bacterial pathogens.

Knowledge of the influence of disease and inflammation on cellular IL-6R and gp130 expression in vivo is highly limited. In patients with HIV infection, enhanced expression of IL-6R has been found on circulating monocytes, B cells, and CD4+ T cells [26]. In rats, acute inflammation induced by various stimuli, including LPS, was associated with enhanced gene expression of IL-6R and gp130 in hepatocytes [27, 28]. Receptor expression at the cell surface was not examined in these studies. We found reduced expression of IL-6R on circulating monocytes and granulocytes, accompanied by unchanged expression of gp130, after LPS administration, suggesting that LPS-induced acute inflammation is associated with a down-regulation...
of only the ligand-binding part of the functional IL-6R complex. In a pathophysiologic way, this finding is not unexpected, because IL-6 responsiveness of cells is determined by IL-6R expression rather than by expression of gp130 [4].

The present data extend earlier findings on the regulation of TNF receptors (TNF-Rs) in normal subjects exposed to LPS and in patients with sepsis, in whom both type I and type II TNF-Rs are down-modulated on monocytes and granulocytes [19, 20, 29, 30]. Reduced TNF-R expression on inflammatory cells upon exposure to infectious pathogens may reflect a mechanism by which the host protects itself against excessive toxicity evoked by this potent proinflammatory cytokine. A pathophysiologic explanation for reduced IL-6R expression seems less clear. Indeed, IL-6 does not cause serious toxicity, even when administered at high doses [31, 32], and a number of studies have documented anti-inflammatory effects of IL-6, including inhibition of TNF production and stimulation of release of soluble TNF and IL-1 inhibitors [33–35]. It should be noted, however, that intravenous LPS and sepsis also cause down-regulation of the type II IL-1 receptor, which serves a purely anti-inflammatory role by functioning as an IL-1 decoy receptor [20, 36]. Together, these data suggest that acute inflammation results in down-modulation of both pro- and anti-inflammatory cytokine receptors at the surface of monocytes and granulocytes.

The LPS-induced decrease in IL-6R expression was not accompanied by an increase in the plasma concentrations of soluble IL-6R. In previous studies, the down-regulation of cellular TNF-R after LPS injection was associated with enhanced release of soluble TNF-R, although a direct correlation between the 2 phenomena could not be demonstrated [19, 20, 30]. In contrast, reduced type II IL-1R expression was not accompanied by increased plasma concentrations of soluble type II IL-1R [20]. It remains to be established whether the down-modulation of IL-6R (and other cytokine receptors) during endotoxemia is the result of shedding or internalization. The unaltered plasma levels of soluble IL-6R may have been related to the relatively mild challenge or to the possibility that putative shedding of IL-6R by circulating monocytes and granulocytes does not induce significant changes in the large pool of soluble IL-6R in the circulation. In addition, this pool may be derived from cells not present in blood. Furthermore, while inflammatory diseases invariably are associated with elevated circulating levels of IL-6, such conditions can result in increased

Figure 5. Lipopolysaccharide (LPS) does not influence monocyte and granulocyte gp130 in whole blood in vitro. Whole blood was incubated for indicated times in presence or absence of LPS (10 ng/mL). Bars show difference between specific mean channel fluorescence (MCF) and nonspecific MCF of 4 different donors (mean ± SE).

Figure 6. Gram-positive stimuli and cytokines induce down-regulation of monocyte and granulocyte interleukin-6 receptor (IL-6R) in whole blood in vitro. Whole blood was incubated 4 h in 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 IL-6 (10 ng/mL), recombinant human interferon (IFN)-γ (10 ng/mL), and recombinant human tumor necrosis factor (TNF; 10 ng/mL). Bars are difference between specific mean channel fluorescence (MCF) and nonspecific MCF of 4 different donors (mean ± SE).
unaltered [37, 38], or decreased [17] plasma levels of soluble IL-6R. Together these data suggest that an elevation in the plasma concentrations of soluble IL-6R is not a uniform reaction to injurious stimuli in vivo. In accordance with our study, soluble gp130 concentrations were unchanged in patients with severe malaria [16].

LIF is a member of the IL-6 cytokine family and shares many biologic activities with IL-6 [4, 39]. LIF binds at low affinity to the LIF-R, after which the LIF-R becomes heterodimerized with gp130 to form a high-affinity and signal-transducing receptor complex [40]. Hence, although the IL-6R functions exclusively to present IL-6 to surface-expressed gp130, the LIF-R is critical for signal transduction itself. In addition, unlike the IL-6R, the structure of the LIF-R is closely related to that of gp130 [41]. In an earlier investigation, LPS injection into rats increased LIF-R mRNA levels in hepatocytes [28]. In our study, we found that the binding of recombinant LIF to circulating monocytes and granulocytes did not change after LPS administration, suggesting that the expression of functional LIF-R/gp130 complexes remained unaltered. LPS also did not induce LIF release into the circulation. Some, but not all, patients with sepsis have elevated LIF concentrations in the circulation [42, 43], whereas LIF is undetectable in patients with malaria [16]. Conceivably, only severe inflammatory stimuli result in detectable LIF in blood. In accordance, in baboons challenged with a lethal dose of live E. coli, LIF plasma levels were much higher than in baboons infected with a sublethal dose of bacteria [44].

IL-6 is an important mediator of the acute phase protein response and has a pivotal role in host defense against various classes of pathogens. We report here that LPS injection into normal subjects is associated with down-regulation of IL-6R expression on peripheral blood monocytes and granulocytes but does not influence gp130 expression or LIF binding. Similar changes were found upon stimulation of whole blood with LPS or gram-positive stimuli in vitro. Thus, monocytes and granulocytes may respond with a reduction in IL-6R expression on their surface shortly after their first interaction with invading bacteria. Further studies are warranted to evaluate the molecular mechanisms and the functional consequences of this response during clinical infection.

References

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