Cytokines and chemokines in systemic and urinary tract infection by Escherichia coli
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Chapter 11

Effect of interleukin (IL-10) on the release of the CXC chemokines growth-regulated oncogene (GRO)-α and epithelial-cell-derived neutrophil-activating peptide (ENA)-78 during human endotoxemia.

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Abstract

Pretreatment with interleukin (IL)-10 inhibited the release of growth-related oncogene (GRO)-α, but not of epithelial-cell derived neutrophil activating protein (ENA)-78, after injection of lipopolysaccharide (LPS) into healthy humans. In vitro, IL-10 dose-dependently attenuated LPS-induced release of both GROα and ENA-78 in whole blood and in cultures of isolated polymorphonuclear and mononuclear cells.
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**Introduction**

Chemokines constitute a family of small chemotactic proteins that orchestrate leukocyte trafficking in response to inflammation and infection (8, 13). CXC chemokines form a subfamily in which the two cysteines nearest the N-termini are separated by one amino acid. CXC chemokines can be further divided into ELR positive CXC chemokines, which possess a three amino acid motif termed ELR (glutamic acid – leucine – arginine) near the N-terminal end, and ELR negative CXC chemokines. ELR positive CXC chemokines are chemotactic for neutrophils, and include interleukin (IL)-8, growth-related oncogene (GRO)-α, β and γ, and epithelial-cell derived neutrophil activating protein (ENA)-78.

IL-10 is an anti-inflammatory cytokine that inhibits the production of a number of proinflammatory cytokines and chemokines upon stimulation of cells in vitro (11). IL-10 exerts strong anti-inflammatory effects in animal models of systemic inflammation, such as endotoxin shock (4, 5). Experiments in rodents have suggested that IL-10 can influence neutrophil recruitment to inflamed organs (15, 19). This anti-inflammatory effect of IL-10 at least in part can be explained by previous findings that IL-10 can inhibit the stimulated production of IL-8 by monocytes and neutrophils in vitro (1, 3, 6, 14, 18), and during human and primate endotoxemia in vivo (12, 16). Knowledge of the effect of IL-10 on other ELR positive CXC chemokines is lacking. Therefore, in the present investigation we sought to determine the effect of IL-10 on the production of GROα and ENA-78 during human endotoxemia in vivo. In addition, we evaluated the effect of IL-10 on endotoxin (lipopolysaccharide, LPS)-induced GROα and ENA-78 release in whole blood, and in cultures of isolated peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells (PMNs).

**Patients and Methods**

Sixteen healthy male subjects (mean ± SE: 23 ± 1 years) were enrolled in this double-blind, cross-over, randomized study. The design and experimental details of this study have been published previously (10, 12). Briefly, each participant was studied on two occasions, separated by a wash-out period of six weeks. On one occasion the subject was challenged with LPS in
combination with placebo, on the other occasion with LPS in combination with recombinant human IL-10. The volunteers were randomized into two groups of eight subjects. Group 1 received either placebo or IL-10 treatment two minutes prior to LPS challenge, group 2 received either placebo or IL-10 treatment one hour after LPS administration. IL-10 (Schering-Plough Research Institute, Kenilworth, NJ) was administered by direct intravenous injection at a dose of 25 µg/kg contralateral to site of blood sample withdrawal. The placebo preparation, containing excipients, was identical in appearance and was administered in an identical manner. The LPS preparation used in this study, endotoxin reference standard lot G, *E. coli* (United States Pharmacopeia Convention Inc., Rockville, MD), was administered over one minute in an ante-cubital vein, contralateral to the administration site of IL-10, at a dose of 4 ng/kg. Blood was drawn from ante-cubital veins by separate venipunctures directly before LPS administration and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 hours thereafter. Serum was stored at -70 °C until assays were performed.

For in vitro experiments whole blood was collected aseptically from 6 healthy subjects, as described previously (10). Anticoagulation was obtained using sterile endotoxin-free heparin (Leo Pharmaceutical Products, Weesp, the Netherlands; final concentration 10 U/ml). PBMCs and PMNs were isolated from peripheral blood, as described previously (10), using Polymorphprep (Nycomed Pharma AS; Oslo, Norway). In these experiments PBMCs and PMNs were resuspended in LPS-free RPMI 1640 (Bio Wittaker, Verviers, Belgium) to the original blood volume from which they were harvested. Following this method, cell counts in these cultures were 1.73 ± 0.30 x 10⁹/ml (PBMCs) and 2.73 ± 0.74 x 10⁹/ml (PMNs). Purity of the cell fractions was checked using a 0.1% eosin stain and was found to be above 99%. Whole blood, isolated PBMCs or PMNs cells were incubated for 24 hours at 37°C in sterile polypropylene tubes (Becton Dickinson) diluted 1:2 in RPMI 1640 in the presence or absence of LPS (from *E.coli* serotype 0111: B4; Sigma, St. Louis, MO; 10 ng/mL). After the incubation, supernatants were collected and stored at −20 °C until assays were performed. GROα and ENA-78 were measured by ELISA's according to the instructions of the manufacturer (R&D Systems, Abingdon, UK). All data are given as means ± SEM. Log-transformed concentrations of chemokines were analyzed in time by one-way analysis of variance (ANOVA). Comparisons between treatment
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Groups were conducted using repeated measures ANOVA. P < 0.05 was considered to represent a significant difference.

Results

Injection of LPS into healthy humans induced a febrile syndrome associated with influenza-like symptoms (10, 12). LPS elicited transient increases in the serum concentrations of GROα and ENA-78, both peaking after 2 hours (Figure 1; both P < 0.001). IL-10 pretreatment, but not IL-10 posttreatment, attenuated LPS-induced GROα release. Peak GROα concentrations in subjects pretreated with IL-10 were 0.74 ± 0.27 ng/mL versus 1.10 ± 0.37 ng/mL in subjects injected with LPS only (P<0.001). By contrast, neither IL-

![Figure 1](image)

**Figure 1.** Mean ± SE serum concentrations of GROα (upper panels) and ENA-78 (lower panels) after intravenous LPS injection (4 ng/kg; t = 0) into healthy humans. Placebo (open circles) or IL-10 (25 µg/kg, filled circles) was given intravenously two minutes before LPS injection (pretreatment) or one hour after LPS injection (posttreatment). P values indicate the difference between treatment groups. NS = nonsignificant.

10 pretreatment nor IL-10 posttreatment influenced the release of ENA-78 into the circulation of subjects exposed to LPS. To obtain insight into which cell type present in the circulation was influenced by IL-10, we determined
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the effects of IL-10 on GROα and ENA-78 release by peripheral blood, isolated PBMCs and PMNs in vitro (Figure 2). In whole blood, IL-10 exerted a strong dose-dependent inhibiting effect on LPS-induced GROα and ENA-78 release. PBMCs turned out to be the main producers of GROα and ENA-78 per mL blood, releasing 9- and 15-fold more of these chemokines.

**Figure 2.** Mean ± SE concentrations of GROα (open triangles) and ENA-78 (filled triangles) in supernatants of whole blood, PBMCs and PMNs stimulated with LPS (10 ng/mL) at 37 °C for 24 hours in the presence of increasing concentrations of IL-10. PBMCs and PMNs were isolated from peripheral blood and resuspended in culture medium to the original blood volume. Data are expressed relative to incubation with LPS alone. Absolute chemokine concentrations measured after LPS stimulation in the absence of IL-10 are given in the text.
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respectively, than PMNs. Concentrations in supernatants of LPS stimulated PBMCs and PMNs were 5.01 ± 1.50 ng/mL and 0.61 ± 0.11 ng/mL respectively for GROα, and 6.53 ± 1.56 ng/mL and 0.45 ± 0.20 ng/mL for ENA-78. IL-10 dose-dependently inhibited GROα and ENA-78 release by LPS stimulated PBMCs and PMNs. Interestingly, IL-10 doses required to maximally attenuate CXC chemokine production by whole blood were 1000-fold lower than IL-10 doses needed to abrogate chemokine release by isolated PBMCs and PMNs. Furthermore, PMNs were more sensitive to the inhibitory effect of IL-10 than PBMCs, with reduced chemokine release already observed at 10 ng/mL whereas 100 ng/mL was necessary to reduce chemokine production by PBMCs.

Discussion

The present study is the first to investigate the effect of IL-10 on the ELR positive CXC chemokines GROα and ENA-78. Unlike IL-8, which can be considered the prototypic member of this class of neutrophil activating peptides, GROα and ENA-78 interact only with the type 2 CXC chemokine receptor (8, 13). Each of these three chemokines likely contributes to neutrophil trafficking during infection and inflammation (9, 13, 17). We therefore considered it of interest to assess the effects of IL-10 on the release of GROα and ENA-78. In vivo, IL-10 given two minutes prior to LPS, inhibited GROα secretion into the circulation, while not altering the release of ENA-78. In vitro, IL-10 proved to be a strong inhibitor of the secretion of both CXC chemokines. The discrepancy between the effect of IL-10 on ENA-78 production in healthy humans in vivo and in whole blood or cultures of isolated blood cells in vitro suggests that during human endotoxemia cell types not present in peripheral blood contribute to ENA-78 release to a more important extent than to the secretion of GROα (and IL-8). Indeed, ENA-78 can be synthesized by many different cell types, including epithelial cells, keratinocytes, smooth muscle cells, endothelial cells and fibroblasts (17). Further, in support of this explanation, IL-10 did not influence LPS-induced IL-8 release by fibroblasts (14) and even enhanced IL-8 production by endothelial cells (2), indicating that the effect of IL-10 on the production of ELR positive CXC chemokines is not uniformly inhibitory. It should be noted that the IL-10 induced inhibition of IL-8 (12) and GROα release (the present study) after in vivo administration of LPS, contrasts with the enhanced
secretion of the ELR negative CXC chemokines gamma interferon-inducible protein (IP)-10 and monokine induced by interferon-γ (Mig) observed in IL-10 treated humans (7). Further studies are warranted to dissect the molecular mechanisms underlying these differential effects of IL-10 on ELR positive and ELR negative CXC chemokines. The present data may contribute to the understanding of the anti-inflammatory potential of IL-10 during neutrophil-mediated diseases.
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