Interleukin 10 inhibits the release of CC chemokines during human endotoxemia


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Interleukin 10 Inhibits the Release of CC Chemokines during Human Endotoxemia

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Sixteen healthy subjects were intravenously injected with lipopolysaccharide (LPS), once with placebo and once with recombinant human interleukin (IL)–10 (25 μg/kg), to determine the effect of IL-10 on LPS-induced production of macrophage inflammatory protein (MIP)–1α, MIP-1β, and monocyte chemotactant protein (MCP)–1. LPS induced transient increases in serum MIP-1α, MIP-1β, and MCP-1. Pretreatment with IL-10 inhibited LPS-induced release of MIP-1α, MIP-1β, and MCP-1. In whole blood in vitro, the IL-10–induced inhibition of MIP-1α and MIP-1β release was equally potent in the presence or absence of an anti–tumor necrosis factor (TNF) antibody. Although isolated peripheral blood mononuclear cells produced more MIP-1α and MIP-1β than neutrophils, the latter cells were more sensitive to the inhibiting effect of IL-10. IL-10 attenuates LPS-induced production of CC chemokines in human endotoxemia, whereby in vitro experiments suggest that, in the case of MIP-1α and MIP-1β release, this effect is independent from an inhibitory effect on TNF production.

Chemokines are a family of small chemotactic proteins that play an important role in inflammatory responses, as mediators of leukocyte trafficking and activation [1, 2]. Chemokines have been divided into several families on the basis of their structure. Macrophage inflammatory protein (MIP)–1α, MIP-1β, and monocyte chemotactant protein (MCP)–1 belong to the CC chemokine family and act primarily on monocytes and macrophages.

Endotoxin is the lipopolysaccharide (LPS) part of the outer membrane of gram-negative bacteria and is considered to be responsible for the induction of inflammation during gram-negative infection. Administration of LPS to healthy humans induces a systemic inflammatory response with many characteristics of sepsis, such as cytokine production, leukocyte activation, and activation of coagulation and fibrinolysis [3]. Furthermore, iv injection of LPS has been found to elicit transient rises in the plasma concentrations of MIP-1α, MIP-1β, and MCP-1 [4, 5]. In accordance, the circulating levels of these CC chemokines were also elevated in patients with sepsis [4, 6, 7].

Interleukin (IL)–10 is a major anti-inflammatory cytokine that potently inhibits production of proinflammatory mediators such as tumor necrosis factor-α (TNF) and IL-1 [8, 9]. The anti-inflammatory properties of IL-10 are enhanced by stimulation of the production of IL-1 receptor antagonist [10] and shedding of TNF receptors [11]. Elevated IL-10 levels have been reported in patients with sepsis [12, 13] and experimental human endotoxemia [13]. In LPS-challenge models, IL-10 appears to have a protective function. Indeed, recombinant IL-10 reduced TNF release in response to LPS and protected against LPS-induced mortality in mice [14]. Neutralization of endogenously produced IL-10 in endotoxemic mice resulted in an increased production of several proinflammatory cytokines, including TNF, and enhanced mortality [15]. Similarly, IL-10 gene–deficient mice demonstrated higher mortality rates after LPS injection [16].

Knowledge of the effect of IL-10 on CC chemokine production is limited to in vitro studies. In these studies, IL-10 was found to inhibit the production of MIP-1α and MIP-1β by murine macrophages [17, 18], as well as LPS-induced production of MIP-1α by human blood monocytes [19] and polymorphonuclear cells [20]. Both stimulatory and inhibitory effects of IL-10 on MCP-1 synthesis in vitro have been described [21–23]. In the present study, we sought to determine the in vivo effect of IL-10 on the production of MIP-1α, MIP-1β, and MCP-1 in the well-established model of human endotoxemia.

Materials and Methods

In vivo study. The in vivo study was done simultaneously with investigations determining the effects of IL-10 on cytokines, gran-
ulocytes, and the hemostatic mechanism, the results of which have been published elsewhere [24, 25]. Sixteen healthy male subjects (mean age ± SE: 23 ± 1 years) were enrolled in this double-blind, crossover, randomized study. Results of medical history, physical and routine laboratory examination, chest radiogram, and electrocardiogram were normal. The subjects did not smoke, did not use any medication, and did not have any febrile illness in the month preceding the start of the study. Each participant was studied on 2 occasions, separated by a washout period of 6 weeks. On one occasion, each subject was challenged with LPS in combination with placebo, on the other occasion, with LPS in combination with recombinant human (rh) IL-10. The volunteers were randomized into 2 groups of 8 subjects. Group 1 received either placebo or rhIL-10 treatment 2 min before LPS challenge; group 2 received either placebo or rhIL-10 treatment 1 h after LPS administration. The study was done in a special research unit under the continuous supervision of at least 2 physicians with emergency and resuscitation equipment available. Blood pressure and heart rate were assessed every 30 min with a Dinamap blood pressure monitor (Criticon, Tampa, FL) during the first 8 h after LPS challenge.

The rhIL-10 (Schering-Plough Research Institute, Kenilworth, NJ) was supplied as a sterile powder. After reconstitution with sterile water, rhIL-10 was administered by direct iv injection at a dose of 25 µg/kg contralateral to the site of blood sample withdrawal. The reconstituted placebo powder, 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, Escherichia coli (United States Pharmacopeia Conversion Inc., Rockville, MD), was administered over 1 min in an antecubital vein, contralateral to the administration site of rhIL-10, at a dose of 4 ng/kg.

Blood was drawn from antecubital veins by separate venipunctures directly before LPS administration and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 h thereafter. Blood for chemokine measurements was collected in nonadditive Vacutainer tubes (Becton Dickinson, Mountain View, CA); after clotting, the samples were centrifuged at 2000 g for 20 min at room temperature, and the resulting serum was stored at −70°C until assays were done.

In vitro experiments. Whole blood stimulation was done as described elsewhere [26]. For each experiment whole blood was collected aseptically from 6 healthy subjects by means of a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson, Rutherford, NJ). Anticoagulation was obtained by use of sterile endotoxin-free heparin (Leo Pharmaeutical Products, Weesp, the Netherlands; final concentration 10 U/mL). Whole blood, isolated peripheral blood mononuclear cells (PBMC) or polymorphonuclear cells (PMN) (see below) were incubated for 24 h in 37°C in sterile polypropylene tubes (Becton Dickinson) diluted 1:2 in endotoxin-free RPMI 1640 (Bio Wittaker, Verviers, Belgium) in the presence or absence of the following reagents: LPS from E. coli serotype 0111: B4 (Sigma, St. Louis, MO; final concentration 10 ng/mL), lipoteichoic acid from Staphylococcus aureus (LTA, Sigma, 1 µg/mL), staphylococcal enterotoxin B (SEB, Sigma, 1µg/mL), heat-killed S. aureus (HKSA, 107 cfu/mL), rhIL-10 (Schering-Plough Research Institute; final concentration 0.01, 0.1, 1, 10, 100, or 1000 ng/mL), rhTNF (10 ng/mL), monoclonal mouse derived anti-human TNF-α antibody (MAK 195F; final concentration 25 µg/mL) [27], or mouse IgG (Sigma; final concentration 25 µg/mL). The amount of MAK 195F added represents an excess neutralizing activity relative to the amount of TNF produced in LPS-stimulated whole blood [27, and data not shown]. Knoll AG (Ludwigshafen, Germany) generously provided rhTNF and MAK 195F. After the incubation, polypropylene tubes were centrifuged at 3000 rpm (rotor diameter, 154 mm) at 4°C for 12 min. Supernatants were collected and stored at −20°C until assays were done.

In separate experiments, PBMC and PMNs were isolated from peripheral blood. For this purpose, heparinized blood was layered on an equal volume of Polymorphprep (Nycemed Pharma AS; Oslo, Norway) and centrifuged at 500 g for 30 min at 20°C. The collected PBMC and PMN fractions were diluted 1:2 in 0.5 N RPMI 1640 to restore normal osmolality and spun at 400 g for 10 min at 20°C. Remaining erythrocytes were lysed by use of ice-cold isotonic NH4Cl solution (155 mM NH4Cl, 10 mM KHCO3, and 0.1 mM EDTA, pH 7.4) for 10 min. The cell fractions were spun again at 400 g for 10 min at 4°C, and the pellet was resuspended in 1N RPMI containing 5% normal human serum (Bio Wittaker, Walkersville, MD) to the original volume. Purity of the cell fractions was checked by means of a 0.1% eosin stain and was found to be more than 99%. After the incubation, supernatants were collected.

Assays. All assays were done in duplicate by enzyme-linked immunosorbent assay (ELISA). For determination of MIP-1α and MIP-1β levels, purified monoclonal mouse anti-human MIP-1α and anti-human MIP-1β were used as coating antibodies (4 µg/mL; R&D Systems, Abingdon, United Kingdom), biotinylated affinity purified goat anti-human MIP-1α and MIP-1β as detecting antibodies (20 ng/mL; R&D Systems), and rhMIP-1α and rhMIP-1β as standards (R&D Systems). MCP-1 was measured with use of purified monoclonal mouse anti-human MCP-1 (2 µg/mL; Pharmingen, San Diego, CA) as coating antibody, biotinylated rabbit anti-human MCP-1 (1 µg/mL; Pharmingen) as detecting antibody, and rhMCP-1 (Pharmingen) as standard. TNF levels were determined by use of monoclonal mouse anti-human TNF (2 µg/mL; Medgenix, Fleurus, Belgium) as coating antibody, biotinylated mouse anti-human TNF (0.5 µg/mL; Medgenix) and rhTNF (Medgenix) as standard. Detection limits of the assays were 15.6 pg/mL (MIP-1α), 15.6 pg/mL (MIP-1β), 8.2 pg/mL (MCP-1) and 6.9 pg/mL (TNF).

Statistical analysis. All data are given as mean ± SEM. Comparisons between treatment groups were done by repeated-measures ANOVA after log transformation of chemokine concentrations. Data obtained in in vitro experiments were compared by Wilcoxon test. The α level for all tests was set at 0.05.

Results

Effect of IL-10 on chemokine production in whole blood in vitro. To determine the effect of IL-10 on LPS-induced production of MIP-1α, MIP-1β, and MCP-1, we incubated human whole blood for 24 h with LPS (10 ng/mL) in the presence or absence of increasing concentrations of rhIL-10. In whole blood incubated without LPS, MIP-1α and MIP-1β levels were undetectable and MCP-1 was 1.70 ± 0.18 ng/mL. IL-10 alone (without LPS) induced production of MCP-1 in whole blood
(3.77 ± 0.46 ng/mL; P < .05 vs. controls), whereas MIP-1α and MIP-1β remained undetectable. LPS stimulated the production of all 3 chemokines (MIP-1α, 42.54 ± 8.46 ng/mL; MIP-1β, 178.67 ± 23.71 ng/mL; MCP-1, 18.26 ± 4.55 ng/mL; all P < .05 vs. controls). IL-10 also dose-dependently inhibited LPS-induced production of MIP-1α and MIP-1β. By contrast, IL-10 did not influence MCP-1 concentrations after stimulation with LPS (figure 1). TNF was undetectable in whole blood incubated without LPS. LPS induced increased levels of TNF (3.05 ± 0.36 ng/mL; P < .05 vs. control). As expected, IL-10 reduced LPS-induced TNF concentrations in a dose-dependent fashion (figure 1). We next determined whether IL-10 also inhibited chemokine production induced by other infectious stimuli. This proved to be the case; that is, IL-10 reduced the production of MIP-1α and MIP-1β by whole blood stimulated with LTA, SEB, or HKSA, although IL-10 exerted the strongest inhibitory effect on chemokine production induced by LPS (table 1). In addition, IL-10 attenuated MIP-1α and MIP-1β release induced by TNF (table 1). The effect of IL-10 on MCP-1 production stimulated by LTA, SEB, HKSA, or TNF was variable and modest (table 1).

Effect of IL-10 on the LPS-induced CC chemokine production in vivo. Having established that IL-10 inhibits the production of certain CC chemokines in whole blood in vitro, we measured the capacity of IL-10 to influence chemokine release in vivo. Therefore, we measured serum concentrations of MIP-1α, MIP-1β, and MCP-1 in healthy humans exposed to a low dose of LPS with or without a single injection of IL-10 given either 2 min before (pretreatment) or 1 h after (posttreatment) LPS. We previously reported that IL-10 pretreatment, but not IL-10 posttreatment, significantly reduced LPS-induced TNF release [25]. LPS administration was associated with a rise in serum MIP-1α concentrations. Peak MIP-1α levels were reached at 1.5–2 h (group 1, 1.74 ± 0.92 ng/mL; group 2, 0.31 ± 0.08 ng/mL; both P < .01). Pretreatment with rhIL-10 attenuated the LPS-induced increase in MIP-1α concentrations to 0.58 ± 0.44 ng/mL (P < .05 vs. placebo), whereas posttreatment tended to reduce MIP-1α release (peak levels, 0.19 ± 0.04 ng/mL; non-significant vs. placebo) (figure 2). LPS injection induced an increase in MIP-1β serum levels, peaking after 2–3 h (group 1, 6.39 ± 1.26 ng/mL; group 2, 5.35 ± 0.48 ng/mL; both P < .001). Both pretreatment and posttreatment with rhIL-10 significantly reduced LPS-induced MIP-1β release, with peak MIP-1β levels of 2.38 ± 0.64 ng/mL (P < .001) and 3.58 ± 0.65 ng/mL (P = .001) respectively (figure 2). Intravenous injection of LPS induced an increase in MCP-1 serum levels, peaking after 3–4 h (group 1, 13.11 ± 1.51 ng/mL; group 2, 15.53 ± 2.66 ng/mL; both P < .001). rhIL-10 pretreatment significantly reduced LPS-induced MCP-1 release, with peak concentrations of only 6.93 ± 1.62 ng/mL (P < .001 vs. placebo);

![Figure 1. IL-10 dose-dependently inhibits lipopolysaccharide (LPS)-induced macrophage inflammatory protein (MIP)-1α, MIP-1β, and tumor necrosis factor (TNF) release without influencing MCP-1 production. Whole blood diluted 1:2 in RPMI was incubated with LPS (10 ng/mL) for 24 h at 37°C with increasing concentrations of rh interleukin (IL)-10. Results are expressed relative to incubation with LPS alone, as means ± SEM (n = 6).](image)

### Table 1. Effect of interleukin (IL)-10 on chemokine production by whole blood stimulated with various infectious stimuli or tumor necrosis factor (TNF).

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>10 ng/mL IL-10 present</th>
<th>MIP-1α, ng/mL</th>
<th>MIP-1β, ng/mL</th>
<th>MCP-1, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS (10 ng/mL)</td>
<td>No</td>
<td>50.78 ± 10.46</td>
<td>143.07 ± 20.96</td>
<td>46.88 ± 8.87</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>5.58 ± 0.53 (–87.1 ± 2.2)</td>
<td>24.38 ± 1.84 (–81.8 ± 1.7)</td>
<td>53.76 ± 5.86 (31.6 ± 23.6)</td>
</tr>
<tr>
<td>LTA (1 μg/mL)</td>
<td>No</td>
<td>43.56 ± 18.56</td>
<td>48.15 ± 10.64</td>
<td>109.41 ± 13.1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>17.88 ± 5.77 (–42.4 ± 14.5)</td>
<td>19.68 ± 3.97 (–54.7 ± 4.3)</td>
<td>92.24 ± 20.82 (–16.77 ± 17.5)</td>
</tr>
<tr>
<td>SEB (1 μg/mL)</td>
<td>No</td>
<td>11.14 ± 2.65</td>
<td>62.87 ± 9.87</td>
<td>127.59 ± 26.65</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>4.19 ± 0.53 (–58.1 ± 3.9)</td>
<td>19.88 ± 1.95 (–65.5 ± 4.1)</td>
<td>105.60 ± 25.66 (–18.6 ± 4.1)</td>
</tr>
<tr>
<td>HKSA (10^7 CFU/mL)</td>
<td>No</td>
<td>53.9 ± 11.86</td>
<td>67.13 ± 9</td>
<td>75.43 ± 11.1</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>25.99 ± 2.56 (–37.3 ± 12.7)</td>
<td>24.37 ± 1.45 (–59.5 ± 6.8)</td>
<td>156.92 ± 58.36 (88.2 ± 44.2)</td>
</tr>
<tr>
<td>TNF (10 ng/mL)</td>
<td>No</td>
<td>0.13 ± 0.03</td>
<td>2.12 ± 0.12</td>
<td>7.95 ± 1.31</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0.09 ± 0.01 (–62.6 ± 4.3)</td>
<td>0.31 ± 0.08 (–78.6 ± 18.4)</td>
<td>6.87 ± 0.15 (–16.6 ± 6.6)</td>
</tr>
</tbody>
</table>

**NOTE.** Data are mean ± SEM of 6 different donors; values in parentheses indicate % change in values from chemokine production in the absence of rhIL-10 to production in its presence. Whole blood diluted 1:2 in RPMI was incubated for 24 h at 37°C with the stimuli indicated. LPS, lipopolysaccharide; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory protein.

* P < .05 vs. incubation in the absence of IL-10.
rhIL-10 posttreatment did not reduce MCP-1 concentrations, which peaked at 16.28 ± 3.09 ng/mL (nonsignificant vs. placebo) (figure 2).

Role of TNF in inhibition of LPS-induced MIP-1α and MIP-1β production by IL-10. It has been well established that IL-10 inhibits LPS-induced TNF production in vivo [14, 15, 25, 28] and in vitro [9, 29–32] (figure 1). Considering that TNF can induce the production of chemokines [33] (table 1), we tried to determine whether IL-10–induced inhibition of TNF production is involved in the reduction of MIP-1α and MIP-1β concentrations in whole blood incubated with LPS and IL-10.

However, neutralization of TNF produced in the whole blood system by coincubation with an anti–TNF monoclonal antibody (mAb) only modestly reduced LPS-induced MIP-1α and MIP-1β release (table 2). Furthermore, the potency by which IL-10 inhibited MIP-1α and MIP-1β production in LPS-stimulated whole blood was similar in the presence or absence of anti–TNF (table 2). Hence, these data suggest that, although endogenous TNF positively influences MIP-1α and MIP-1β release induced by LPS, IL-10 exerts its inhibitory effect on the production of these chemokines by a mechanism that does not involve inhibition of TNF synthesis.

Figure 2. Mean ± SEM macrophage inflammatory protein (MIP)-1α, MIP-1β, and monocyte chemoattractant protein (MCP)-1 concentrations after lipopolysaccharide (LPS) administration (4 ng/kg) to healthy humans. Placebo (○) or rh interleukin (IL)-10 (25 μg/kg iv, ●) was given just before LPS challenge (pretreatment) or 1 h after endotoxin administration (posttreatment). P-values indicate difference between treatment groups. NS, nonsignificant. Note that the Y axes for MIP-1α differ in pre- and posttreatment groups.
**Table 2.** Effect of interleukin (IL)-10 on LPS-induced production of macrophage inflammatory protein (MIP)-1α and MIP-1β in human whole blood in the presence or absence of anti–tumor necrosis factor (TNF).

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>MIP-1α, ng/mL</th>
<th>MIP-1β, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS (10 ng/mL)</td>
<td>37.72 ± 5</td>
<td>145.33 ± 22.63</td>
</tr>
<tr>
<td>Positive</td>
<td>1.82 ± 0.32 (−94.7 ± 1.2)</td>
<td>7.18 ± 1.65 (−94.2 ± 1.7)</td>
</tr>
<tr>
<td>LPS (10 ng/mL) + anti-TNF (27 µg/mL)</td>
<td>30.42 ± 4.69</td>
<td>113.57 ± 17.18</td>
</tr>
<tr>
<td>Positive</td>
<td>1.65 ± 0.15 (−93.7 ± 1.4)</td>
<td>5.08 ± 1.05 (−95.2 ± 1.1)</td>
</tr>
</tbody>
</table>

*NOTE.* Data are mean ± SEM of 6 different donors; values in parentheses indicate % change in values from chemokine production.

*Significant difference (*P* < .05) from incubation without IL-10.

**Effect of IL-10 on LPS-induced MIP-1α and MIP-1β production by isolated PBMC and PMNs in vitro.** To determine the cellular target of IL-10 in whole blood, we compared the effect of IL-10 on LPS-induced MIP-1α and MIP-1β production by isolated PBMC and PMNs. In these experiments PBMC and PMNs were resuspended in medium to the original blood volume from which they were collected. Using this method, we found that PBMC counts in these cultures were 1.73 (± 0.30) × 10⁷/mL and PMN counts were 2.73 (± 0.74) × 10⁶/mL. PBMC turned out to be the main producers of MIP-1α and MIP-1β per milliliter blood, releasing 20- and 5-fold more of these chemokines, respectively, than did PMNs. For MIP-1α, the concentration in supernatants of stimulated PBMCs was 25.39 ± 6.54 ng/mL and of PMNs, 1.40 ± 0.78 ng/mL; for MIP-1β, the concentration of PMBCs was 15.87 ± 2.05 ng/mL and of PMNs, 3.29 ± 0.96 ng/mL. IL-10 at 10 ng/mL (a concentration that strongly inhibited chemokine production in whole blood) attenuated LPS-induced MIP-1α and MIP-1β release by PMNs (both *P* < 0.05), but not by PBMC. In cultures of isolated PBMC, an IL-10 concentration of 100 ng/mL was required to significantly reduce MIP-1α and MIP-1β release (both *P* < 0.05) (figure 3).

**Discussion**

Endotoxemia and gram-negative sepsis are associated with elevated levels of the CC chemokines MIP-1α, MIP-1β and MCP-1 in the circulation. Previous work has established that IL-10 serves a potent anti-inflammatory role during endotoxemia, primarily through inhibition of the production of proinflammatory cytokines, such as TNF. The present study is the first to demonstrate the in vivo capability of IL-10 to inhibit the release of MIP-1α, MIP-1β, and MCP-1. Administration of rhIL-10 directly before LPS injection into healthy humans strongly attenuated the secretion of all 3 chemokines; rhIL-10 administration postponed for 1 h relative to LPS injection reduced only MIP-1β release. This IL-10 effect could be reproduced in part in whole blood in vitro, that is, IL-10 reduced LPS-stimulated production of MIP-1α and MIP-1β, but not of MCP-1, by a mechanism that was independent of IL-10-induced TNF inhibition. In separate experiments, PBMC turned out to be the main producers of MIP-1α and MIP-1β in whole blood, but PMNs were most sensitive to the inhibiting effect of IL-10.

MIP-1α levels were higher in group 1 than in group 2 (see figure 2). This difference is likely a reflection of interindividual variation, because all subjects were studied according to an identical protocol, and all MIP-1α measurements were conducted in one run. The differential MIP-1α responses could not have influenced our analysis, because the study was done in a crossover design in which each subject served as his own control.

The effects of IL-10 on CC chemokine production have been investigated only in in vitro experiments. IL-10 was reported to reduce the production of MIP-1α by murine bone marrow–derived macrophages infected with *Listeria monocytogenes* [17], and to attenuate MIP-1α and MIP-1β release by primary mouse macrophages stimulated with the extracellular matrix component hyaluronan [18]. By using LPS as a stimulus, we found that IL-10 inhibited MIP-1α production by human monocytes [19] and mouse macrophages [34], although it did not influence MIP-1β production [34]. In cultures of LPS-stimulated PMNs, however, IL-10 was found to inhibit both MIP-1α and MIP-1β release [20]. In the present study, we primarily used whole blood as in vitro system, thereby eliminating possible artifacts associated with isolation of cells, such as adherence-induced expression of TNF [35, 36], and allowing investigations of IL-10 effects in a physiological environment [26, 36]. In this system, IL-10 caused a dose-dependent inhibition of LPS-induced MIP-1α and MIP-1β production, which was similar to the inhibition of LPS-induced TNF production by IL-10. IL-10 also attenuated MIP-1α and MIP-1β production in whole blood incubated with stimuli derived from *S. aureus*, that is, LTA (a cell wall component), SEB (a superantigen), and heat-killed whole bacteria, although IL-10 had the strongest inhibitory effect on MIP-1α and MIP-1β release induced by LPS.

TNF is an important proinflammatory cytokine that mediates activation of various mediator systems during a systemic inflammatory response syndrome such as that provoked by iv administration of LPS. Indeed, neutralization of endogenously produced TNF strongly diminished LPS-induced cytokine release in humans and nonhuman primates [37, 38]. Considering that, first, TNF can induce the production of MIP-1α and MIP-
Figure 3. Mean ± SEM macrophage inflammatory protein (MIP)-1α and MIP-1β concentrations in supernatants of PBMC and PMNs stimulated with lipopolysaccharide (LPS) in the presence of different concentrations of rh interleukin (IL)-10. PBMC and PMNs were isolated from peripheral blood and resuspended in RPMI to the original (blood) volume. Data are expressed relative to incubation with LPS alone. Asterisks indicate significant difference from incubation with LPS alone (P < .005).

1β [39] (table 1), second, IL-10 inhibits LPS-induced TNF production [9, 29, 30, 32] (figure 1), and third, IL-10 inhibits TNF-induced MIP-1α and MIP-1β production (table 1), we found it of interest to determine the contribution of IL-10−induced inhibition of TNF release to the IL-10 effects on MIP-1α and MIP-1β secretion in whole blood. Therefore, to eliminate the effect of reduced TNF concentrations in the presence of IL-10, we did experiments with a neutralizing anti−TNF mAb. Anti−TNF only modestly reduced LPS-induced MIP-1α and MIP-1β production in whole blood, and, in the presence of anti−TNF, IL-10 reduced LPS-induced MIP-1α and MIP-1β production to a similar extent as in the absence of anti−TNF. Together these data suggest that IL-10 influences MIP-1α and MIP-1β production by a mechanism that is independent from its negative effect on TNF production. In accord with a recent study in human volunteers, infusion of a recombinant TNF receptor fusion protein did not influence LPS-induced release of MIP-1α or MIP-1β, although the results of that study must be interpreted with caution because the infusion of the TNF receptor fusion was associated with several paradoxical, unexplained proinflammatory effects [4].

Chemokines can be produced by a variety of immune and nonimmune cells, including blood leukocytes, endothelial cells, and fibroblasts [40−42]. We investigated which cell types were involved in MIP-1α and MIP-1β production in whole blood and determined the effect of IL-10 on isolated PBMC and PMNs. On a per milliliter of blood basis, but also on a per cell basis, PBMC were by far the main producers of both MIP-1α and MIP-1β, which is in keeping with earlier findings that PMNs produce fewer cytokines than PBMC on stimulation with LPS [43]. Interestingly, higher IL-10 concentrations were required to inhibit MIP-1α and MIP-1β production by PMNs than by PBMC. Previously, differential sensitivities for IL-10 were reported in human blood monocytes and alveolar macrophages [19]. Furthermore, our data suggest that PBMC are more sensitive to IL-10 in their physiological environment (i.e., whole blood) than after their isolation.

MCP-1 is a prototypic CC chemokine with chemotactic activity for mononuclear cells. During endotoxemia, however, MCP-1 may have an anti-inflammatory role [44]. Indeed, passive immunization of mice with anti−MCP-1 antiserum increased LPS-induced mortality, whereas treatment with recombinant MCP-1 protected mice from lethality. Moreover, anti−MCP-1 reduced LPS-induced IL-10 release when compared with animals treated with LPS and control antiserum, whereas recombinant MCP-1 enhanced IL-10 secretion [44]. We now report that pretreatment with rhIL-10 reduced LPS-induced MCP-1 release in humans in vivo. Together these data suggest that MCP-1 stimulates the production of IL-10 in vivo, which in turn can exert a negative feedback effect on ongoing MCP-1 release. However, the effect of IL-10 on LPS-induced MCP-1 production could not be reproduced in whole blood in vitro. Possibly, during human endotoxemia cell types not present in peripheral blood, such as endothelial cells [45] or vascular smooth muscle cells [46], contribute to MCP-1 release into the circulation. In addition, although IL-10 has been reported to inhibit production of MCP-1 by human monocytes stimulated with either LPS or IL-1β, monocytes and alveolar macrophages have been found to release MCP-1 on exposure to IL-10 without additional stimulus [22, 23]. In our hands, IL-10 by itself also induced MCP-1 release in whole blood.

Chemokines have recently been recognized as a large family of low−molecular-weight chemotactic cytokines that play an important role in the pathogenesis of several inflammatory and
infectious diseases. We have demonstrated that a major anti-inflammatory cytokine IL-10 attenuates production of the CC chemokines MIP-1α, MIP-1β, and MCP-1 during human endotoxia. IL-10 has been shown to possess important protective effects in many models of systemic and local inflammation. The present data, taken together with earlier findings that IL-10 can inhibit the production of the prototypic CXC chemokine IL-8 [20, 25, 30], suggest that IL-10 effects may in part relate to its influence on chemokine production.

References