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Attenuation of Proinflammatory Response by Recombinant Human IL-10 in Human Endotoxemia

Effect of Timing of Recombinant Human IL-10 Administration¹

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To determine the effects of IL-10 on cytokine and granulocyte responses during endotoxemia, two groups of eight healthy male volunteers were challenged with endotoxin (4 ng/kg) on two occasions, once in combination with placebo injection, and once in conjunction with i.v. administered recombinant human IL-10 (rhIL-10) (25 µg/kg). In group 1, rhIL-10 was administered 2 min before endotoxin challenge; in group 2, the intervention was delayed for 1 h after endotoxin administration. rhIL-10 pretreatment reduced the LPS-induced rises in temperature and release of TNF, IL-6, IL-8, and IL-1 receptor antagonist. Endotoxin-induced granulocyte accumulation in lungs, as determined by dynamic granuloscintigrams, was prevented by rhIL-10 pretreatment, whereas granulocyte recruitment in liver and spleen was only modestly reduced. In addition, granulocyte degranulation, as measured by plasma elastase/α₁-antitrypsin complexes, was blunted significantly by rhIL-10 pretreatment. Post-treatment with rhIL-10 did not influence LPS-induced temperature responses, cytokine release, or granulocyte degranulation. Both rhIL-10 pretreatment and post-treatment reduced LPS-induced cortisol levels. These results indicate that pretreatment with rhIL-10 reduces endotoxin-induced febrile responses, cytokine responses, and granulocyte accumulation in lungs, while in this acute model post-treatment with rhIL-10 exerts limited anti-inflammatory effects. *The Journal of Immunology*, 1997, 158: 3971–3977.

Cytokines are considered to be of importance for the orchestration of inflammatory responses during sepsis. Cytokines interact in a complex network that consists of proinflammatory and anti-inflammatory mediators. IL-10 is an anti-inflammatory cytokine produced by Th0 and Th2 cells, monocytes/macrophages, and B cells (1–3) that can inhibit the endotoxin-induced production of proinflammatory cytokines by mononuclear cells and granulocytes in vitro (4–8). In addition, IL-10 can enhance the release of IL-1 receptor antagonist (IL-1ra)³ (9), which further contributes to its anti-inflammatory properties. These effects of IL-10 on cytokine release result in significant protection against lethality in mouse models of endotoxic shock (10, 11). Evidence exists that IL-10 can also attenuate tissue injury mediated by activated neutrophils. IL-10 abrogated lung injury during immune complex-induced alveolitis in rats (12), whereas anti-IL-10 potentiated inflammatory effects within the lung in this

model (13). Current knowledge of the potential anti-inflammatory effects of IL-10 in humans is limited. Administration of rhIL-10 to humans caused suppression of the production of TNF and IL-1β by whole blood after ex vivo stimulation with endotoxin, while the production of soluble TNFR p55 and IL-1ra remained unchanged (14, 15).

We designed a crossover, placebo-controlled, randomized study in two groups of healthy volunteers to determine the effect of rhIL-10, administered i.v. immediately before or 1 h after injection of endotoxin, on endotoxin-induced clinical symptoms, alterations in vital signs, cytokine release, granulocyte degranulation, and granulocyte recruitment into lungs, liver, and spleen.

Materials and Methods

Study group and design

Sixteen healthy male volunteers (mean 23, range 20–35 yr) were enrolled in this double-blind, crossover, randomized, placebo-controlled study. Written informed consent was obtained from all study subjects. Medical history, physical and routine laboratory examination, chest x-ray, and electrocardiogram were normal. The volunteers 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 two occasions, separated by a washout period of 6 wk. On one occasion, the subject was challenged with endotoxin in combination with placebo; on the other, in combination with rhIL-10.

The volunteers were randomized into two groups of eight subjects. Group 1 received either placebo or rhIL-10 treatment 2 min before endotoxin challenge; group 2 received either placebo or rhIL-10 treatment 1 h after endotoxin administration. The study was approved by the research and ethical committees of Academic Medical Center.

The study was performed at a special research unit under the continuous supervision of at least two physicians, with emergency and resuscitation equipment immediately available.

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³ Abbreviations used in this paper: IL-1ra, interleukin-1 receptor antagonist; HMPAO, hexamethylpropyleneamineoxime; LAL, *Limulus* amoebocyte lysate; rh, recombinant human.

Blood pressure and heart rate were assessed every 30 min using a Dinamap blood pressure monitor (Criticon, Tampa, FL) during the first 8 h after endotoxin challenge; oral temperature and respiratory rate were assessed at the same time points. Adverse events were registered throughout the confinement periods.

rhIL-10 (Schering-Plough Research Institute, Kenilworth, NJ) was supplied as a sterile powder. After reconstitution with sterile water, the appropriate volume of rhIL-10 was administered by direct i.v. injection at a dose of 25 $\mu\text{g}/\text{kg}$ contralateral to site of blood sample withdrawal. The reconstituted placebo powder, containing excipients, was identical in appearance and was administered in an identical manner.

The endotoxin preparation used in this study, endotoxin reference standard lot G, *Escherichia coli* (United States Pharmacopeia Convention, Rockville, MD), was administered in 1 min in an antecubital vein, contralateral to the administration site of rhIL-10, at a dose of 4 ng/kg . The endotoxin preparation used in this study exerts similar endotoxin activity as lot EC-5, *E. coli* (Food and Drug Administration, Bethesda, MD), as determined by *Limulus* amoebocyte lysate (LAL) assay (data not shown).

Blood sampling

Blood was drawn from antecubital veins by separate venipunctures directly before endotoxin administration, and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 12, and 20 h thereafter. Additional blood sampling for endotoxin activity assays was performed at 5, 10, and 15 min after endotoxin administration. For leukocyte and differential counts, blood was collected in tubes containing EDTA (K_3) (15%) and was counted by flow cytometry (Technicon HI system; Technicon Instruments, Tarrytown, NY). Blood for endotoxin measurements was collected in pyrogen-free plastic tubes (Falcon 2063, Oxnard, CA) containing pyrogen-free heparin (Thromboliquine Organon, Oss, The Netherlands; final concentration 30 IU/ml). Blood for cytokine measurements was collected in nonadditive vacutainer tubes (Becton Dickinson, Mountain View, CA); after clotting, the samples were centrifuged at $2000 \times g$ for 20 min at room temperature, and the resulting serum was stored at -20°C until assays were performed. Blood for determination of elastase/ α_1 -antitrypsin complexes (elastase) was collected in tubes containing 10 mmol/L EDTA, 10 mmol/L benzamidine hydrochloride hydrate 98%, and 100 $\mu\text{g}/\text{ml}$ soybean trypsin inhibitor. Blood for the determination of cortisol concentrations was drawn in heparinized tubes (Becton Dickinson).

Assays

For the LAL assay, platelet-rich plasma was prepared by centrifugation of heparinized blood at $180 \times g$ for 10 min at 4°C and subsequently stored at -20°C . The *Limulus* assay was performed as described previously (16). Inhibitors and activated clotting factors were removed by dilution and heating at 37°C for 5 min. Standard curves were made with *E. coli* O55: B5 (Mallinckrodt, St. Louis, MO). This assay had a detection limit in plasma of 36 EU/L. Each sample was measured in duplicate, and the results were expressed as the mean of the two tests.

Cytokine concentrations were determined using specific ELISAs (TNF, Medgenix Diagnostic, Brussels, Belgium; IL-1ra, Quantikine, R & D Systems, Abingdon, U.K.; IL-6 and IL-8, Central Laboratory of The Netherlands Red Cross Blood Transfusion Service CLB, Amsterdam, The Netherlands; and IL-10, Schering-Plough Research Institute). Elastase was measured by RIA (17); cortisol concentrations were determined by fluorescence polarization immunoassay (Abbott, Amstelveen, The Netherlands).

Radiolabeling of granulocytes

All procedures were performed in a laminar flow chamber using sterile, pyrogen-free glassware and instruments. Five hours before endotoxin administration, 100 ml of venous blood was collected from the volunteers from group 1 into 20% (v/v) acid citrate dextrose (3.2%) and 6% (v/v) hydroxyethyl ether starch (Fresenius, 's Hertogenbosch, The Netherlands). After sedimentation at room temperature for 45 min, the supernatant, composed of leukocyte-rich plasma, was centrifuged at $200 \times g$ for 10 min, and the pellet was resuspended in 3 ml of autologous plasma. The granulocytes were isolated by Percoll density-gradient centrifugation (Pharmacia, Woerden, The Netherlands) at $200 \times g$ for 30 min. The pellet, containing pure granulocytes, was suspended in 10 ml of saline, centrifuged at $200 \times g$ for 10 min to remove the Percoll, and labeled with 925 mBq of $^{99\text{m}}\text{Tc}$ -HMPAO (hexamethylpropyleneamineoxime) (Amersham, Hertogenbosch, The Netherlands), as previously described (18). After an incubation period of 20 min, the labeled granulocytes were centrifuged at $200 \times g$ for 10 min to remove free $^{99\text{m}}\text{Tc}$ -HMPAO. The remaining pellet, consisting of $^{99\text{m}}\text{Tc}$ -HMPAO-labeled granulocytes, was brought up to a volume of 2 ml with

saline and administered i.v. 2 h before endotoxin administration. The final total dose administered varied from 164 to 580 mBq.

Dynamic granuloscintigrams

Scintigraphic images were recorded continuously from 5 min before endotoxin administration until 120 min after endotoxin administration. Dynamic anterior views from the thorax/upper abdomen were obtained with a single-head large field-of-view gamma camera (Diacam, Siemens, Gammasonics, Hoffman Estates, IL) fitted with a low energy collimator. Images were digitized and stored (Hermes, Nuclear Diagnostics, Hagersten, Sweden) in a 128×128 matrix. The evaluation included time-activity curves derived from regions of interest over the lungs, liver, and spleen. Granulocyte recruitment to the lungs, liver, and spleen was expressed as a decay-corrected percentage of the pre-endotoxin values starting at 100%.

Statistical analysis

Data are presented as mean \pm SEM. Differences between the placebo and the rhIL-10 treatment groups were tested by crossover ANOVA for repeated measures, using SPSS for Windows. Changes of variables over time were analyzed using one-way ANOVA. The p values quoted in the text indicate the total treatment effects (two-way ANOVA) or total time effects (one-way ANOVA). A two-sided p value ≤ 0.05 was considered significant.

Results

Clinical symptoms, safety parameters, and vital signs

Endotoxin administration elicited similar clinical symptoms as previously described (19, 20). Briefly, influenza-like signs and symptoms were observed, including headache, chills, nausea, vomiting, myalgia, and fever. All clinical adverse events resolved within 20 h after endotoxin challenge.

There was no difference in severity and incidence between placebo and rhIL-10 treatment periods of the endotoxin-induced flu-like symptoms. In group 1, endotoxin-induced rises in temperature with peak mean levels of $38.3 \pm 0.2^\circ\text{C}$ were reduced by rhIL-10 treatment to $37.8 \pm 0.1^\circ\text{C}$ ($p = 0.04$ vs placebo); in group 2, from peak mean levels of $37.9 \pm 0.2^\circ\text{C}$ to $37.7 \pm 0.2^\circ\text{C}$ ($p = 0.7$ vs placebo). No differences were found in heart rate, systolic and diastolic, and mean arterial blood pressure between placebo or rhIL-10 treatment periods (data not shown).

Endotoxin activity in plasma

Peak plasma endotoxin levels (104.1 ± 16.1 EU/L, group 1; 142.8 ± 17.4 EU/L, group 2) were observed 5 min after endotoxin injection in both groups. rhIL-10 treatment did not have an effect on endotoxin activity, as measured by the LAL assay (data not shown).

Cytokine concentrations

Administration of rhIL-10 caused an increase in mean serum IL-10 levels to 250.99 ± 11.23 ng/ml (group 1) and 280.60 ± 31.77 ng/ml (group 2) at 10 min after rhIL-10 administration, which rapidly decreased to undetectable concentrations within 4 h after rhIL-10 administration. The administration of endotoxin in conjunction with placebo caused a modest increase in IL-10 serum concentrations that peaked at $t = 3$ h: 0.11 ± 0.06 ng/ml (group 1) and 0.14 ± 0.04 ng/ml (group 2). As shown in Figure 1A, endotoxin administration increased serum TNF levels up to 2.85 ± 0.77 ng/ml (group 1) and 3.22 ± 0.81 ng/ml (group 2) at $t = 2$ h. rhIL-10 pretreatment significantly reduced peak mean TNF levels, resulting in peak concentrations of 0.66 ± 0.11 ng/ml at $t = 1.5$ h ($p < 0.05$ vs placebo); rhIL-10 post-treatment modestly attenuated TNF concentrations to 2.49 ± 0.48 ng/ml at $t = 1.5$ h ($p = 0.35$). Endotoxin/placebo infusion caused an increase in IL-6 levels to peak values of 5.69 ± 3.02 ng/ml (group 1, $t = 3$ h) and $4.16 \pm$

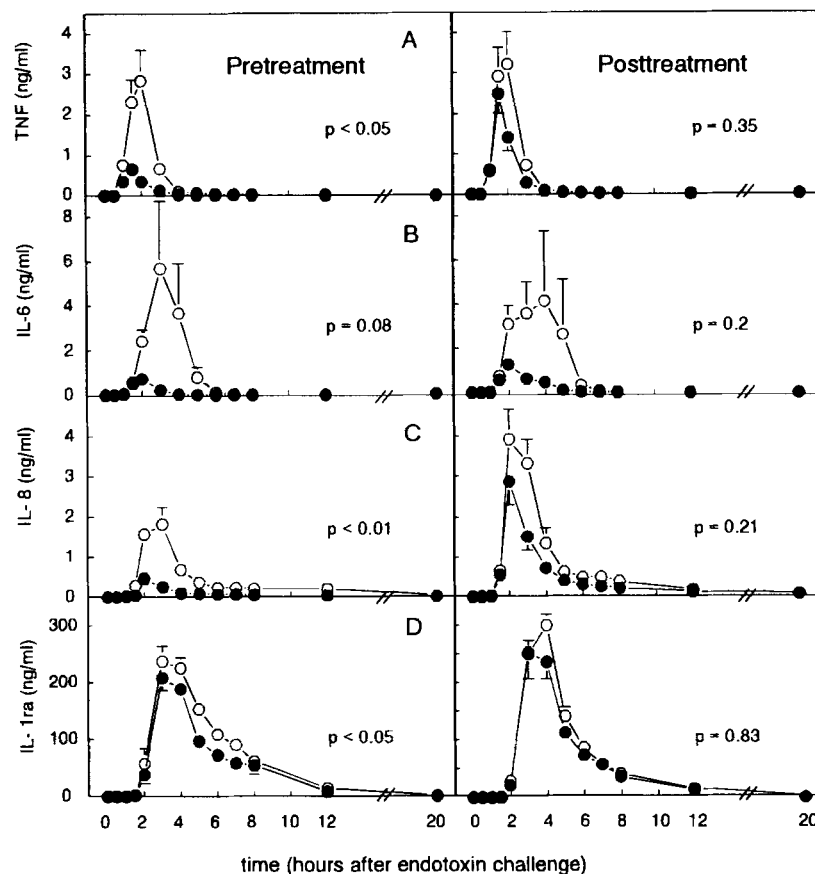


FIGURE 1. Mean (\pm SEM) TNF (A), IL-6 (B), IL-8 (C), and IL-1ra (D) concentrations after endotoxin administration (4 ng/kg) in humans. Placebo (open circles) or rhIL-10 (25 μ g/kg i.v., solid circles) was given just before endotoxin challenge (pretreatment) or 1 h after endotoxin administration (post-treatment). p values indicate differences between treatment groups.

3.13 ng/ml (group 2, $t = 4$ h) ($p < 0.01$ vs time). Pretreatment with rhIL-10 reduced LPS-induced IL-6 levels, with peak levels of 0.73 ± 0.21 ng/ml ($p = 0.08$ vs placebo); rhIL-10 post-treatment tended to inhibit LPS-induced IL-6 release (peak levels of 1.29 ± 0.23 ng/ml; at $t = 2$ h ($p = 0.22$)) (Fig. 1B). rhIL-10 pretreatment reduced serum levels of IL-8 from peak levels of 1.83 ± 0.42 ng/ml at $t = 3$ h (LPS/placebo) to peak levels of 0.47 ± 0.11 ng/ml at $t = 2$ h ($p < 0.01$ vs placebo); rhIL-10 post-treatment tended to reduce LPS-induced increase in IL-8 serum concentrations from peak concentrations of 3.92 ± 0.73 ng/ml to 2.87 ± 0.58 ng/ml at $t = 2$ h ($p = 0.21$) (Fig. 1C).

rhIL-10 pretreatment reduced serum levels of IL-1ra from peak levels of 237.38 ± 26.18 ng/ml at $t = 3$ h (LPS/placebo) to peak levels of 208.13 ± 22.38 ng/ml at $t = 3$ h ($p < 0.05$ vs placebo); rhIL-10 post-treatment had no effect on LPS-induced increases in IL-1ra serum levels ($p = 0.83$) (Fig. 1D).

Leukocyte counts

After endotoxin challenge, mean leukocyte counts decreased from $5.1 \pm 0.5 \times 10^9/L$ (group 1) and $5.8 \pm 0.8 \times 10^9/L$ (group 2) to $3.4 \pm 0.5 \times 10^9/L$ (group 1, $t = 1$ h) and $3.6 \pm 0.5 \times 10^9/L$ (group 2, $t = 1.5$ h), which was followed by an increase up to $14.6 \pm 1.7 \times 10^9/L$ (group 1) and $13.7 \pm 1.1 \times 10^9/L$ (group 2) at $t = 8$ h (Fig. 2A). rhIL-10 did not influence the early leukopenia, but the subsequent leukocytosis was reduced by both rhIL-10 pre- and post-treatment ($p < 0.05$ and $p = 0.08$ vs placebo, respectively, ANOVA from $t = 4$ h). Peak leukocyte counts in rhIL-10-treated subjects were $10.4 \pm 0.8 \times 10^9/L$ (group 1, $t = 7$ h) and $11 \pm 1.1 \times 10^9/L$ (group 2, $t = 6$ h). Biphasic changes in total leukocyte counts were largely caused by changes in neutrophil counts. Like

the effects on total leukocytes, rhIL-10 pre- and post-treatment did not influence the initial neutropenia, but blunted the subsequent neutrophilia (Fig. 2B).

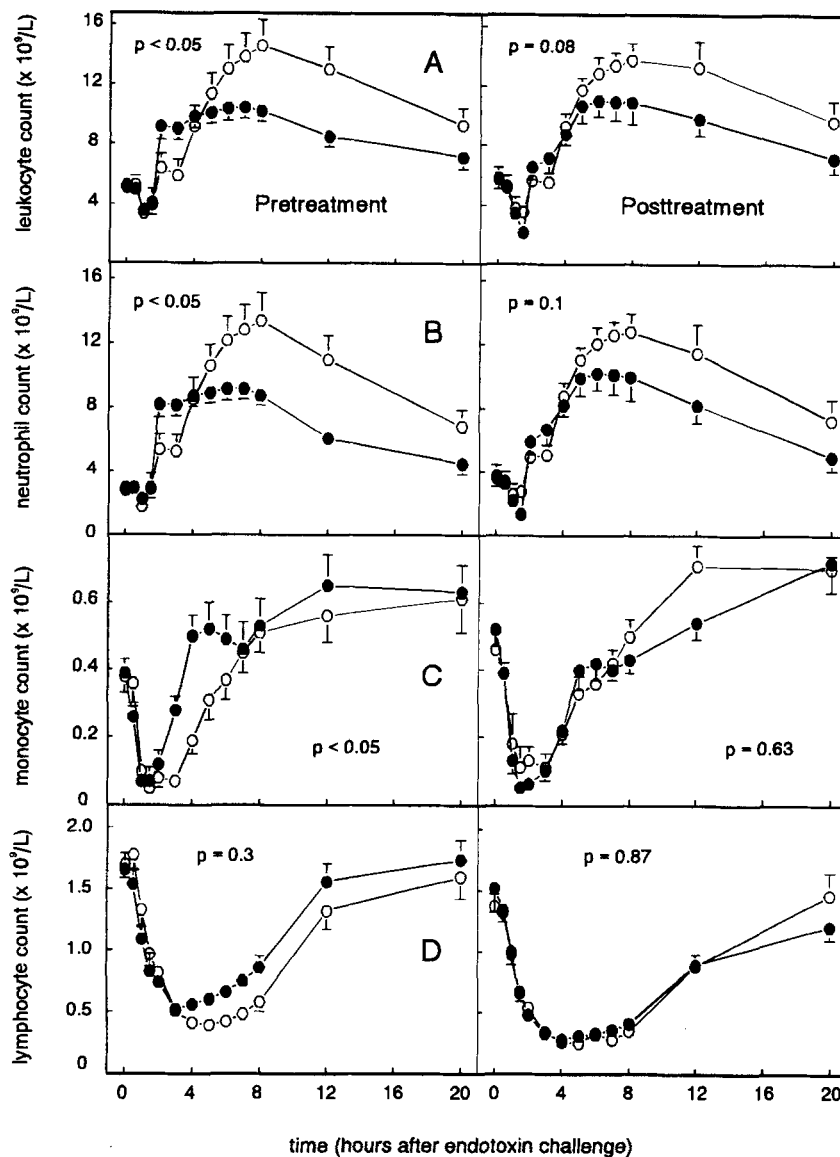
Endotoxin/placebo treatment caused a fall in monocyte counts from baseline levels of $0.38 \pm 0.05 \times 10^9/L$ (group 1) and $0.46 \pm 0.05 \times 10^9/L$ (group 2) to $0.05 \pm 0.01 \times 10^9/L$ (group 1) and $0.11 \pm 0.06 \times 10^9/L$ (group 2) at $t = 1.5$ h, followed by a rise in monocyte numbers to $0.61 \pm 0.10 \times 10^9/L$ (group 1) and $0.70 \pm 0.04 \times 10^9/L$ (group 2) at $t = 20$ h. rhIL-10 pretreatment attenuated the endotoxin-induced changes in monocyte counts ($p < 0.05$ vs placebo), while rhIL-10 post-treatment had no such effect ($p = 0.63$ vs placebo) (Fig. 2C).

Neither rhIL-10 pretreatment nor post-treatment had a significant effect on the LPS-induced changes in lymphocyte counts ($p = 0.3$, $p = 0.87$ vs placebo, respectively) (Fig. 2D).

Dynamic granuloscintigrams

Endotoxin administration resulted in an increase in radioactivity in the lungs up to $141.8 \pm 13.7\%$ at $t = 105$ min ($p < 0.01$ vs time; Fig. 3). This increase of radiolabeled granulocytes into the lungs was blocked completely by rhIL-10 ($107 \pm 8.8\%$, $p < 0.05$ vs placebo). Accumulation of radiolabeled granulocytes in the liver peaked at 115 min after endotoxin administration to $156.5 \pm 6.5\%$ ($p < 0.01$ vs time). rhIL-10 pretreatment, however, did not significantly influence the endotoxin-induced increase in radioactivity in the liver ($149.7 \pm 12.7\%$, $p = 0.07$ vs placebo). Similarly, endotoxin-induced neutrophil accumulation in the spleen region ($p < 0.01$ vs time) was not affected significantly by rhIL-10 pretreatment ($p = 0.07$ vs placebo).

FIGURE 2. Mean (\pm SEM) leukocyte counts in human endotoxemia. Total leukocyte counts (A), neutrophils (B), monocytes (C), and lymphocytes (D). Placebo (open circles) or rhIL-10 (25 μ g/kg i.v., solid circles) was given just before endotoxin challenge (pretreatment) or 1 h after endotoxin challenge (post-treatment). *p* values indicate differences between treatment groups.



Granulocyte degranulation and cortisol levels

Endotoxin infusion caused increases in elastase concentrations from 30.1 ± 3.4 ng/ml to 171.8 ± 26.1 ng/ml (group 1), and from 34.8 ± 6.8 ng/ml to 161.9 ± 33 ng/ml (group 2) at $t = 4$ h. rhIL-10 pretreatment reduced the endotoxin-induced elastase release (100.6 ± 18.1 ng/ml at $t = 4$ h; $p < 0.01$ vs placebo). rhIL-10 post-treatment did not have any effect on elastase levels ($p = 0.52$) (Fig. 4).

As shown in Figure 5, both rhIL-10 pre- and post-treatment significantly blunted endotoxin-induced elevations in cortisol levels. In group 1, cortisol was reduced from 1.06 ± 0.06 μ mol/L ($t = 4$ h) to 0.78 ± 0.06 μ mol/L ($t = 3$ h, $p < 0.01$ vs placebo), and in group 2, from 1.09 ± 0.02 μ mol/L ($t = 4$ h) to 0.88 ± 0.03 μ mol/L ($t = 3$ h, $p < 0.01$ vs placebo).

Discussion

The results from this study demonstrate that pretreatment with rhIL-10 reduced the LPS-induced increases in pro- and anti-inflammatory cytokines and neutrophil degranulation, and prevented accumulation of granulocytes within the lungs in humans.

Delayed rhIL-10 administration (1 h after endotoxin administration) did affect neither LPS-induced cytokine release nor neutrophil degranulation. rhIL-10 pretreatment, but not post-treatment, attenuated the febrile response, and neither pretreatment nor post-treatment had any effect on other endotoxin-induced changes in vital signs or clinical symptoms. Finally, both treatments with rhIL-10 significantly inhibited LPS-induced elevations in cortisol concentrations.

The dose of rhIL-10 used in our study was based on results from ex vivo studies, demonstrating that IL-10 dose dependently reduced the release of TNF and IL-1 β in LPS-stimulated whole blood (14, 15). Thus, the maximally tolerated dose reported in the earlier human studies was used in our investigation, and levels of circulating IL-10 in our study were similar to those found earlier (15).

In line with a number of in vitro reports (1–5), pretreatment with rhIL-10 strongly reduced the LPS-induced release of TNF and IL-8. The lack of significance in reducing IL-6 release by IL-10 pretreatment is most likely due to the intersubject variability in endotoxin response found in this group of volunteers. As is shown

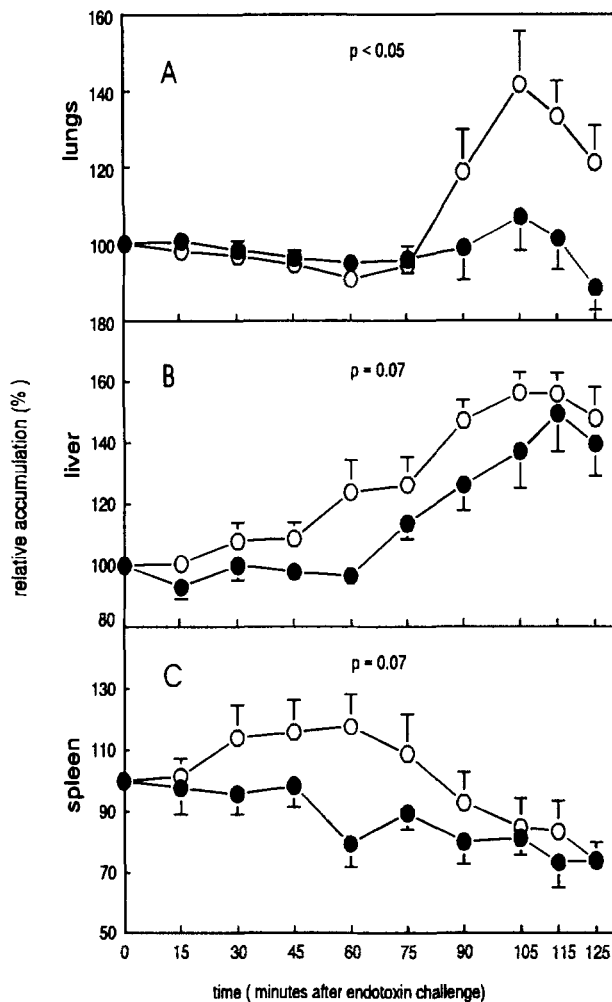


FIGURE 3. Mean (\pm SEM) of relative radioactivity in lungs (A), liver (B), and spleen (C) after endotoxin challenge in humans (4 ng/kg); placebo (open circles) or rhIL-10 (25 μ g/kg i.v., solid circles) was given just before endotoxin administration. *p* values indicate differences between treatment groups.

in Figure 1B, IL-10 pretreatment almost completely prevented IL-6 release. In previous *ex vivo* experiments in humans, IL-10 did not influence IL-1ra release in response to LPS (14, 15); however, in our study, endotoxin-induced elevations in IL-1ra levels were reduced by rhIL-10 pretreatment, whereas post-treatment with IL-10 had no effect on IL-1ra concentrations. These results are in line with a previous study, demonstrating that IL-1ra release in endotoxemia is mediated by TNF (21). It thus seems likely that TNF is a more potent stimulus for IL-1ra release *in vivo* than IL-10, as pretreatment with IL-10 reduced TNF release significantly, while having a moderate effect on TNF levels when administered 1 h after LPS challenge.

rhIL-10 strongly influenced leukocyte responses during endotoxemia. rhIL-10 pretreatment potently reduced the endotoxin-induced neutrophilia and monopenia. It is assumed that the early neutropenia seen during endotoxemia is caused by a retention of these cells within the microcirculation in specific organs. This retention is caused by specific interactions of adhesion molecules and their ligands or by aspecific stiffening of the neutrophil microskeleton (22, 23). Using dynamic granuloscintiscans, we detected an increase in radioactivity over the lungs, liver, and spleen regions within the first 2 h after endotoxin challenge. In accor-

dance with previous animal studies of neutrophil-mediated lung injury (12, 13), rhIL-10 pretreatment significantly reduced endotoxin-induced granulocyte accumulation in the lungs, but had only moderate effects in the liver and spleen. As reported elsewhere,⁴ endotoxin-induced granulocyte accumulation in the lungs in humans can be affected by the expression of L-selectin on circulating neutrophils. Furthermore, in animal models of endotoxemia, blocking CD18 expression on neutrophils was associated with reduced leukocyte accumulation and lung injury (24). Since in the present study expression of adhesion molecules on circulating granulocytes were not determined, we can only speculate that IL-10 may have reduced endotoxin-induced granulocyte accumulation in the lungs by influencing the expression of these adhesion molecules. An explanation for the differences in blockade of granulocyte accumulation by rhIL-10 in different organs could be that the pulmonary vasculature is of much smaller diameter than in liver and spleen, causing a physical blockade of small diameter microcapillaries, with subsequent retention of endotoxin-induced stiffened granulocytes (23). Because of the fact that endotoxin induces neutrophil stiffness within a couple of minutes (23), it seems unlikely that granulocyte accumulation is caused by direct effects of LPS on neutrophil stiffening. The time course of granulocyte accumulation in the lungs resembles the LPS-induced release of TNF, and it therefore can be speculated that other mediators than LPS itself are of importance for granulocyte entrapment *in vivo* in humans. It should be noted that LPS increased granulocyte accumulation within the lungs only during the second hour after LPS challenge, which coincides with the nadir of peripheral neutrophils. Although rhIL-10 did not affect the neutropenic phase, rhIL-10 prevented neutrophil accumulation within the lungs, indicating that low peripheral blood neutrophil counts do not automatically indicate increased granulocyte accumulation within the lungs. IL-10 attenuates granulocyte activation *in vitro* (7, 8), and in our experiments rhIL-10 pretreatment reduced elastase levels. In contrast, elastase concentrations were not influenced when rhIL-10 was administered 1 h after endotoxin challenge. These data suggest that the reduction of neutrophil degranulation by rhIL-10 may have been a consequence of the reduction of TNF release, rather than a direct effect on neutrophil degranulation (25, 26). Indeed, in a previous study, TNF caused an increase in plasma elastase concentrations in humans, and anti-TNF treatment inhibited this response in endotoxemic chimpanzees (25, 26).

Endotoxemia causes an increase of the concentration of circulating glucocorticosteroids. Endogenous cortisol release is considered to represent a counter-regulatory mechanism, as cortisol can inhibit the release of proinflammatory cytokines (27–29), while enhancing IL-10 release (30). rhIL-10 significantly reduced endotoxin-induced cortisol elevations, a finding that contrasts with a previous study in mice, in which no effect of IL-10 was observed (31). Conceivably, interspecies differences in IL-10 responses and/or sensitivity play a role in this apparent discrepancy.

In conclusion, rhIL-10, at a dose of 25 μ g/kg administered 2 min before endotoxin challenge, reduced the release of TNF, IL-6, IL-8, and IL-1ra, blunted temperature responses, and attenuated cortisol release in human endotoxemia. rhIL-10 pretreatment blocked endotoxin-induced granulocyte recruitment in the lungs and granulocyte activation. Administration of rhIL-10 1 h after

⁴ Pajkrt, D., A. Manten, T. van der Poll, M. C. M. Tiel van Buul, J. W. ten Cate, and S. J. H. van Deventer. Modulation of cytokine release and neutrophil function by granulocyte colony-stimulating factor during human endotoxemia. Submitted for publication.

FIGURE 4. The effect of rhIL-10 (25 $\mu\text{g}/\text{kg}$ i.v.) on mean (\pm SEM) elastase concentrations in human endotoxemia. Placebo (open circles) or rhIL-10 (solid circles) was given just before endotoxin challenge (pretreatment) or 1 h after endotoxin challenge (post-treatment). p values indicate differences between treatment groups.

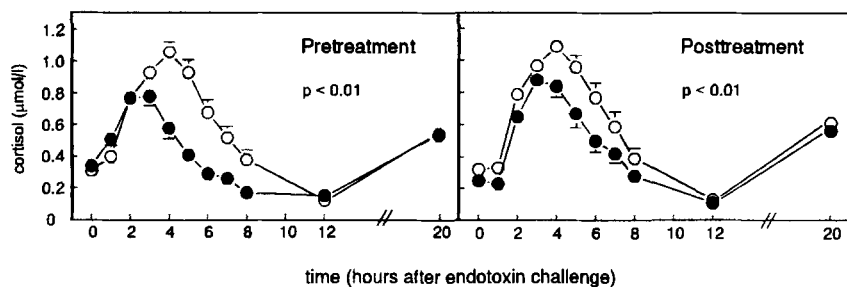
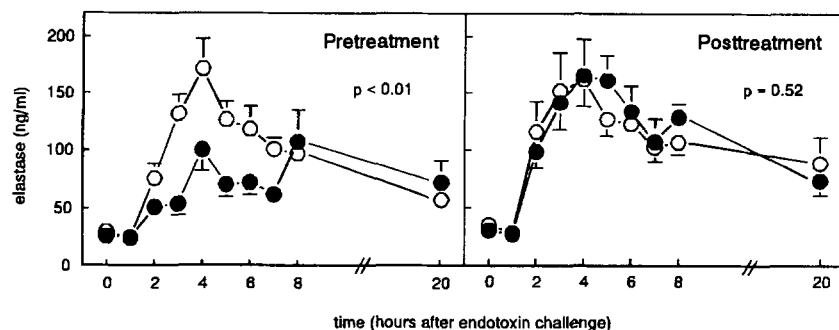


FIGURE 5. Mean (\pm SEM) cortisol concentrations after endotoxin injection (4 ng/kg) in humans. The effect of placebo (open circles) in comparison with rhIL-10 (25 $\mu\text{g}/\text{kg}$ i.v., solid circles); placebo/rhIL-10 was administered either 2 min before endotoxin challenge (pretreatment) or 1 h after endotoxin challenge (post-treatment). p values indicate differences between treatment groups.

endotoxin infusion had no inhibitory effect on LPS-induced cytokine release or granulocyte activation, but still reduced LPS-elicited elevations in plasma cortisol. The potential role of exogenous IL-10 in the treatment of patients with sepsis remains to be established, considering the modest effects of rhIL-10 post-treatment and the fact that IL-10 may impair antibacterial defense mechanisms of the host (32).

Acknowledgments

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