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Proinflammatory Effects of IL-10 During Human Endotoxemia¹

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Sander J. H. van Deventer,* and Tom van der Poll*[†]

IL-10 is considered a potent antiinflammatory cytokine that strongly inhibits the production of proinflammatory cytokines. Recent studies have suggested that IL-10 also has immunostimulatory properties on CD4⁺, CD8⁺ T cells, and/or NK cells, resulting in increased IFN- γ production. To determine the effect of IL-10 on IFN- γ production and related inflammatory responses in humans, 16 healthy subjects received a bolus i.v. injection of LPS (4 ng/kg) in combination with either placebo or recombinant human IL-10 (25 μ g/kg), administered just before or 1 h after LPS. IL-10 treatment, particularly when administered after LPS, enhanced LPS-induced IFN- γ release, as well as the release of the IFN- γ -dependent chemokines IFN- γ -inducible protein-10 and monokine induced by IFN- γ , while inhibiting or not influencing the production of IFN- γ -inducing cytokines. In addition, IL-10 treatment enhanced activation of CTLs and NK cells after LPS injection, as reflected by increased levels of soluble granzymes. These data indicate that high-dose IL-10 treatment in patients with inflammatory disorders can be associated with undesired proinflammatory effects. *The Journal of Immunology*, 2000, 165: 2783–2789.

Interleukin-10 is a noncovalently linked homodimeric cytokine that is produced by a large variety of cells, including monocytes/macrophages, B and T lymphocytes, and NK cells (1, 2). IL-10 has many antiinflammatory and immunosuppressive activities. In vitro, IL-10 down-regulates monocyte/macrophage effector functions including Ag-presenting capacity and the production of cytokines such as TNF, IL-1 β , IL-6, IL-8, and IL-12 (3, 4). IL-10 also inhibits T cell proliferation and cytokine production (5). In mice, IL-10 protects against lethality during endotoxemia and staphylococcal enterotoxin B (SEB)³-induced shock (6–8). In addition, IL-10 treatment inhibits the activation of the cytokine network during experimental endotoxemia in primates (9) and humans (10).

Recently, it has been suggested that IL-10 has immunostimulatory properties. In vitro, IL-10 stimulates proliferation and differentiation of activated human B cells (11), and preincubation of resting CD4⁺ lymphocytes with IL-10 enhances their capacity to produce cytokines after activation (12). Moreover, IL-10 enhances IL-2-driven proliferation of preactivated human-purified CD8⁺ T cells (13). Addition of IL-10 to mouse splenic NK cell cultures stimulated with IL-12 and IL-18 results in enhanced IFN- γ pro-

duction (14). In mice, IL-10 injections accelerated graft-vs-host disease and graft rejection in bone marrow recipients, probably mediated by augmented IFN- γ production by T cells (15).

These data indicate that, under certain conditions, IL-10 can have stimulatory effects on CD4⁺, CD8⁺ T cells, and/or NK cells, which may result in increased IFN- γ production. Knowledge of potential proinflammatory effects of IL-10 in humans in vivo is highly limited. Such knowledge is important, because IL-10 has been advocated as a new treatment modality for several diseases, including Crohn's disease and rheumatoid arthritis (16, 17). Therefore, we studied the effect of IL-10 on IFN- γ production and related inflammatory responses during human endotoxemia, a well accepted model of systemic inflammation in humans.

Materials and Methods

Study design

This study was performed simultaneously with investigations on the effects of recombinant human (rh)IL-10 on cytokine production, leukocytes, and the hemostatic mechanism, the results of which have been reported elsewhere (10, 18). A total of 16 healthy volunteers (mean 23, range 20–35 years) participated in a double-blind, cross-over, randomized, placebo-controlled study. Written informed consent was obtained from all study participants. The study was approved by the research and ethical committees of the Academic Medical Center. Medical history, physical and routine laboratory examination, chest x-ray, and electrocardiogram of all volunteers were normal. Each volunteer was studied on two occasions after endotoxin (LPS) injection, separated by a wash-out period of 6 wk; on one occasion in combination with placebo, on the other occasion in combination with rhIL-10. The participants were randomized into two groups of eight persons. Group 1 received placebo or rhIL-10 treatment 2 min before LPS, group 2 received placebo or rhIL-10 1 h after LPS administration.

rhIL-10 (Schering-Plough Research Institute, Kenilworth, NJ) was supplied as a sterile powder and reconstituted with sterile water. rhIL-10 was administered by i.v. injection at a dose of 25 μ g/kg. The reconstituted placebo powder was identical in appearance and was administered in an identical manner. The LPS preparation, LPS reference standard lot G, *Escherichia coli* (United States Pharmacopeia Convention, Rockville, MD) was administered at a dose of 4 ng/kg over 1 min in an ante-cubital vein, contralateral to the site of rhIL-10.

Blood samples were drawn directly before LPS injection and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, and 12 h thereafter. Blood was collected in nonadditive vacutainer tubes (Becton Dickinson, Mountain View, CA); after clotting,

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³ Abbreviations used in this paper: SEB, staphylococcal enterotoxin B; rh, recombinant human; IP-10, IFN- γ -inducible protein-10; Mig, monokine induced by IFN- γ ; GrA, granzyme A; GrB, granzyme B.

samples were centrifuged at $2000 \times g$ for 20 min at room temperature, and serum was stored at -70°C until assays were performed.

Whole blood stimulation

In separate experiments, whole blood was collected aseptically from six healthy donors using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson). Anticoagulation was obtained using LPS-free heparin (Leo Pharmaceutical Products, Weesp, The Netherlands; final concentration 10 U/ml blood). Whole blood, diluted 1:1 in pyrogen-free RPMI 1640 (BioWhittaker, Verviers, Belgium), was stimulated for 24 h at 37°C with LPS (final concentration 10 ng/ml; from *E. coli* serotype 0111:B4; Sigma, St. Louis, MO) in the presence or absence of increasing concentrations of rhIL-10 (0.01–1000 ng/ml; Schering-Plough). In addition, the effect of IL-10 on LPS-induced IFN- γ production was studied in the presence or absence of an anti-IL-12 and/or anti-IL-18 mAb (both mouse IgG, R&D Systems, Abingdon, U.K.; final concentration both 10 $\mu\text{g/ml}$). The concentrations of mAbs represent at least a 1–2 log-unit excess neutralizing capacity over IL-12 and IL-18 concentrations detected after stimulation with LPS (information on the neutralizing capacities of the mAbs used provided by the manufacturer). Control mouse IgG (R&D Systems) was used in the appropriate concentrations. In separate experiments, IL-10 (10 ng/ml) was added at 0.5, 1, 2, or 4 h after the addition of LPS to whole blood. The effect of IL-10 on IFN- γ production was also studied during whole blood stimulation with anti-CD3/anti-CD28 (1:1000; Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB), Amsterdam, The Netherlands), the superantigen SEB (1 $\mu\text{g/ml}$; Sigma), or PHA (5 $\mu\text{g/ml}$; HA16; Murex Diagnostics, Dartford, U.K.). After the incubation, supernatant was obtained after centrifugation and stored at -20°C until assays were performed.

Assays

All measurements were done in duplicate using specific ELISAs. Serum levels of IL-12p70 and IL-12p40 were measured as described previously (19). In short, IL-12 p70 was measured using anti-IL12 p70 mAb 20C2 as coating Ab, biotinylated anti-IL-12 p40 mAb C8.6 as detecting Ab, and rhIL-12 as standard (detection limit 6 pg/ml). IL-12p40 was measured identically to the IL-12p70 ELISA using anti-IL-12p40 mAb C11.79 as coating Ab (detection limit 54 pg/ml). The IL-12p40 ELISA recognizes the total amount of p40, i.e., complexed as IL-12 heterodimer or as p40 monomer. 20C2 was kindly provided by Dr. M. K. Gately (Hoffmann-La Roche, Nutley, NJ); C8.6 and C11.79 were kindly donated by Dr. G. Trinchieri (The Wistar Institute, Philadelphia, PA). IL-18 was measured as described previously (Fujisaki Institute, Okayama, Japan; detection limit 10 pg/ml) (20). IFN- γ (CLB; detection limit 2.4 pg/ml), IL-15, and IFN- γ -inducible protein-10 (IP-10) (both R&D Systems; 8.2 and 20 pg/ml,

respectively), and monokine induced by IFN- γ (Mig) (PharMingen, San Diego, CA; 8.2 pg/ml) were measured according to the instructions of the manufacturer. Levels of soluble granzyme A (GrA) and GrB were measured by specific ELISAs exactly as described previously (21).

Statistical analysis

All data are expressed as mean \pm SE. Changes in time were analyzed by one-way ANOVA (p value vs time). Differences between the placebo and the rhIL-10 treatment groups were analyzed by cross-over ANOVA for repeated measures (p value vs placebo). Data of the in vitro experiments were analyzed by Wilcoxon test. $p < 0.05$ was considered to represent a significant difference.

Results

Effect of IL-10 on serum levels of IFN- γ and IFN- γ -inducing cytokines

Administration of LPS induced a transient elevation of IFN- γ serum concentrations peaking after 4–5 h (4.8 ± 1.0 pg/ml in group 1, 12.8 ± 7.8 pg/ml in group 2; both $p < 0.05$ vs time) (Fig. 1). Remarkably, IL-10 administration enhanced the release of IFN- γ , which was more pronounced for IL-10 posttreatment, resulting in peak levels of 14.4 ± 3.6 pg/ml (group 1) and 28.5 ± 7.4 pg/ml (group 2) (both $p < 0.001$ vs placebo).

The production of IFN- γ is regulated by a coordinated action of a number of monocyte/macrophage-derived cytokines (22). IL-12 is a potent inducer of IFN- γ production, whereas IL-18 synergistically enhances IL-12 effects on IFN- γ synthesis (23–25). In addition, IL-15 and TNF have been identified as costimuli for optimal IFN- γ production (26, 27). Having established that IL-10 enhances LPS-induced IFN- γ release in vivo, we next determined the effect of IL-10 on IFN- γ -inducing cytokines.

IL-12p70 levels remained undetectable after LPS administration in all but two volunteers. Neither IL-10 pretreatment nor IL-10 posttreatment influenced IL-12p70 levels (data not shown). LPS injection resulted in a transient increase in IL-12p40 concentrations peaking at 3 h after LPS injection (group 1: 1.53 ± 0.35 ng/ml, group 2: 1.47 ± 0.23 ng/ml; both $p < 0.001$ vs time) (Fig. 1). IL-10 pretreatment completely prevented the LPS-induced increase of IL-12p40 levels ($p < 0.001$ vs placebo), whereas IL-10

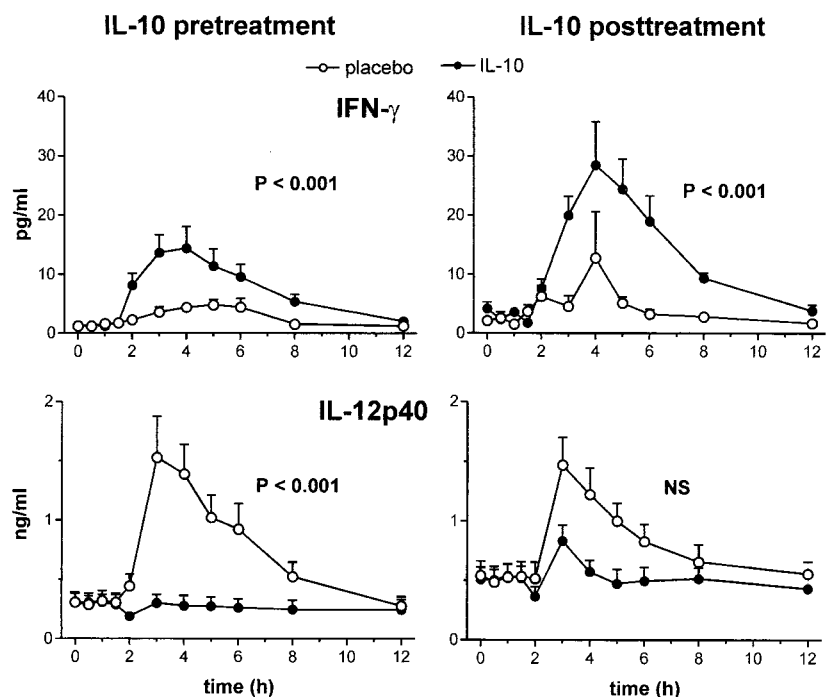


FIGURE 1. IL-10 treatment enhances IFN- γ release during human endotoxemia while reducing IL-12p40 concentrations. Placebo or rhIL-10 (25 $\mu\text{g/kg}$, i.v.) was administered just before (pretreatment) or 1 h after (posttreatment) LPS challenge (4 ng/kg, i.v.). Data are mean \pm SE of eight volunteers in each treatment group. The p value indicates the difference between treatment groups.

posttreatment only partly reduced peak levels of IL-12p40 (0.83 ± 0.13 ng/ml; NS). Serum concentrations of IL-18 were detectable before LPS injection (group 1: 290 ± 68 pg/ml, group 2: 234 ± 45 pg/ml). Administration of LPS did not result in changes in IL-18 levels in either group (data not shown). Also, IL-10 treatment did not influence IL-18 concentrations. IL-15 levels were not detectable before LPS administration and remained undetectable after LPS injection with or without IL-10 treatment. As reported previously, IL-10 pretreatment, but not posttreatment, inhibited LPS-induced TNF release (10). Hence, the stimulatory effect of IL-10 on IFN- γ release was not associated with a detectable increase in IFN- γ -inducing cytokines.

Effect of IL-10 on levels of the IFN- γ -dependent chemokines IP-10 and Mig

To determine whether the IL-10-induced increase in IFN- γ levels also resulted in increased IFN- γ activity, we measured serum concentrations of IP-10 and Mig, CXC chemokines of which the production is largely IFN- γ dependent (28). LPS injection caused a transient increase in IP-10 peaking after 5 h (group 1: 3.47 ± 0.88 ng/ml, group 2: 3.23 ± 0.70 ng/ml; both $p < 0.001$ vs time) (Fig. 2). Although IL-10 pretreatment did not affect IP-10 levels, IL-10 posttreatment resulted in enhanced IP-10 release with peak levels of 4.77 ± 1.19 ng/ml ($p < 0.001$ vs placebo). LPS administration caused increased levels of Mig, which remained elevated until the end of the study period (12 h: group 1: 2.23 ± 0.41 ng/ml, group 2: 2.36 ± 0.58 ng/ml; both $p < 0.001$ vs time). IL-10 pretreatment reduced the early increase in Mig levels between 4–6 h after LPS injection ($p < 0.05$ vs placebo), whereas levels at 12 h were not different. In contrast, IL-10 posttreatment resulted in increased levels of Mig from 6 h until the end of the 12-h study period (4.45 ± 0.74 ng/ml; $p < 0.05$ vs placebo).

Effect of IL-10 on levels of soluble granzymes

We also studied whether IL-10 treatment was associated with activation of CD8⁺ CTLs and/or NK cells, as has been suggested in previous *in vitro* studies (13, 14). Therefore, we measured serum levels of granzymes, proteins that are released from granules of

activated CTL and NK cells (29). LPS injection induced a transient increase in serum levels of GrA, peaking after 1.5–3 h (group 1: 30.1 ± 4.2 pg/ml, group 2: 26.2 ± 5.3 pg/ml; both $p < 0.05$ vs time) (Fig. 3). IL-10 pretreatment did not influence the early rise in GrA levels, but resulted in increased levels of GrA from 6 h after LPS until the end of the 12-h study period ($p < 0.001$ vs placebo). IL-10 posttreatment slightly delayed and increased peak levels of GrA (28.2 ± 2.5 pg/ml), and levels remained elevated until the end of the study period ($p < 0.01$ vs placebo).

GrB levels did not show a significant increase after LPS administration. However, both IL-10 pre- and posttreatment caused a strong increase in GrB levels, peaking after 4–6 h (group 1: 34.8 ± 6.4 pg/ml, group 2: 51.1 ± 14.2 pg/ml; both $p < 0.05$ vs placebo).

Effect of IL-10 on LPS-induced production of IFN- γ and IFN- γ -inducing cytokines *in vitro*

In an attempt to study the mechanisms involved in IL-10-induced enhancement of IFN- γ production, we determined the effect of IL-10 during whole blood stimulations with LPS, an *in vitro* system that is considered to most closely resemble the *in vivo* situation, leaving interactions between different cell populations and serum factors intact (30). Incubation of whole blood for 24 h without stimulus did not result in detectable levels of IFN- γ , IL-12p70, or IL-15, whereas low levels of IL-18 (35.2 ± 5.0 pg/ml) were measured. Incubation with IL-10 alone did not change these levels (data not shown). LPS stimulated the production of IFN- γ (14.9 ± 4.2 ng/ml), IL-12p70 (38.1 ± 13.2 pg/ml), and IL-18 (80.8 ± 7.7 pg/ml) (all $p < 0.05$ vs incubation without LPS), whereas IL-15 remained undetectable. Addition of IL-10 resulted in a strong dose-dependent inhibition of LPS-induced IFN- γ release (IL-10 1 ng/ml: $2.8 \pm 0.4\%$ of IFN- γ levels measured after incubation without IL-10; $p < 0.05$) (Fig. 4A). In addition, IL-10 inhibited the production of IL-12p70 and IL-18 with similar potency (both $p < 0.05$) (Fig. 4A). In the *in vivo* experiments, the enhancement of IFN- γ release occurred in the absence of detectable changes in IL-12p70 and IL-18 levels. In whole blood *in vitro*, the IL-10-induced reduction in IL-12p70 and IL-18 concentrations, which is

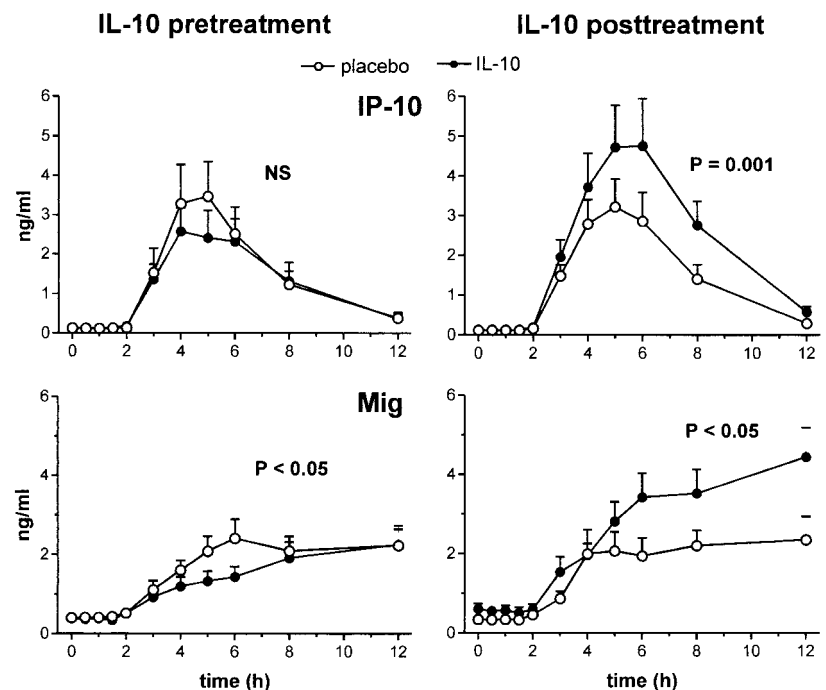
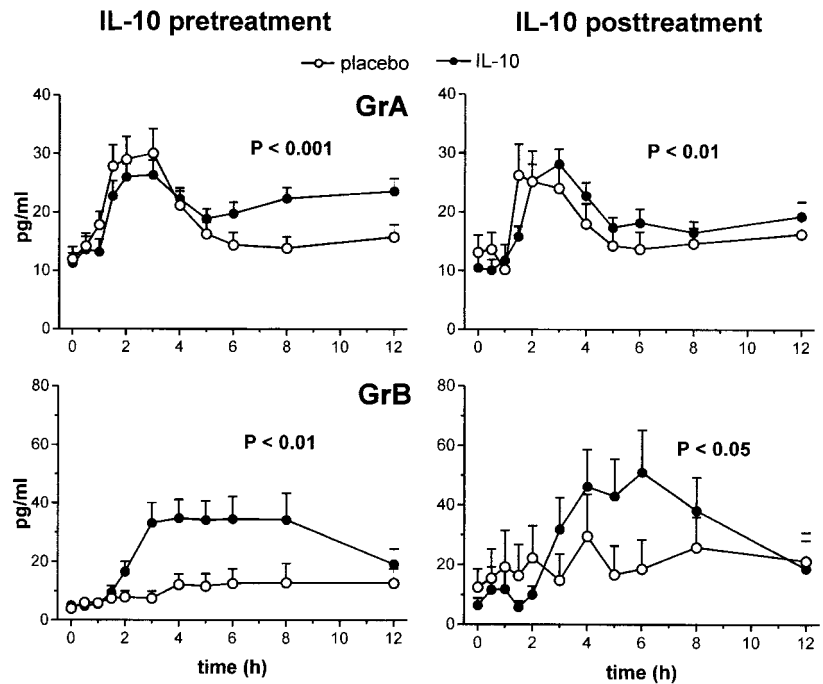


FIGURE 2. Effect of IL-10 treatment on serum levels of the CXC chemokines IP-10 and Mig. LPS was administered in combination with placebo or IL-10 just before (*pretreatment*) or 1 h after (*posttreatment*) LPS. Data are mean \pm SE of eight volunteers in each treatment group. The p value indicates the difference between treatment groups.

FIGURE 3. IL-10 treatment enhances serum levels of GrA and GrB during human endotoxemia. LPS was administered in combination with placebo or rhIL-10 just before (*pretreatment*) or 1 h after (*posttreatment*) LPS. Data are mean \pm SE of eight volunteers in each treatment group. The *p* value indicates the difference between treatment groups.



expected to result in a strong inhibition of IFN- γ release, could have masked a more direct stimulating effect of IL-10 on IFN- γ production. Therefore, we were interested in determining the effect of IL-10 on IFN- γ production in whole blood in the absence of IL-12 and/or IL-18 activity. For this purpose, we performed whole blood stimulations with LPS and IL-10 in the presence or absence of an anti-IL-12 mAb and/or an anti-IL-18 mAb. Although addition of a control Ab did not have any effect, addition of anti-IL-12 or anti-IL-18 resulted in a strong inhibition of IFN- γ release, which was most pronounced for anti-IL-12 (Fig. 4B; both $p < 0.05$). The combination of anti-IL-12 and anti-IL-18 resulted in an additional inhibitory effect ($p < 0.05$ vs anti-IL-12 only). In the

presence of anti-IL-12 or anti-IL-18, IL-10 still inhibited LPS-induced IFN- γ production, although this inhibition was significantly less in the presence of anti-IL-12 (Fig. 4C). The inhibitory effect of IL-10 was further reduced when both anti-IL-12 and anti-IL-18 were added ($p < 0.05$).

Because posttreatment with IL-10 during human endotoxemia resulted in a stronger enhancement of IFN- γ release, we also studied the effect of IL-10 added at different time points after the start of incubation of whole blood with LPS. Addition of IL-10 at 0.5, 1, 2, or 4 h after LPS still potently inhibited IFN- γ production, although this effect was less strong when IL-10 was added 4 h after LPS (data not shown). The inhibitory effect of IL-10 in vitro was

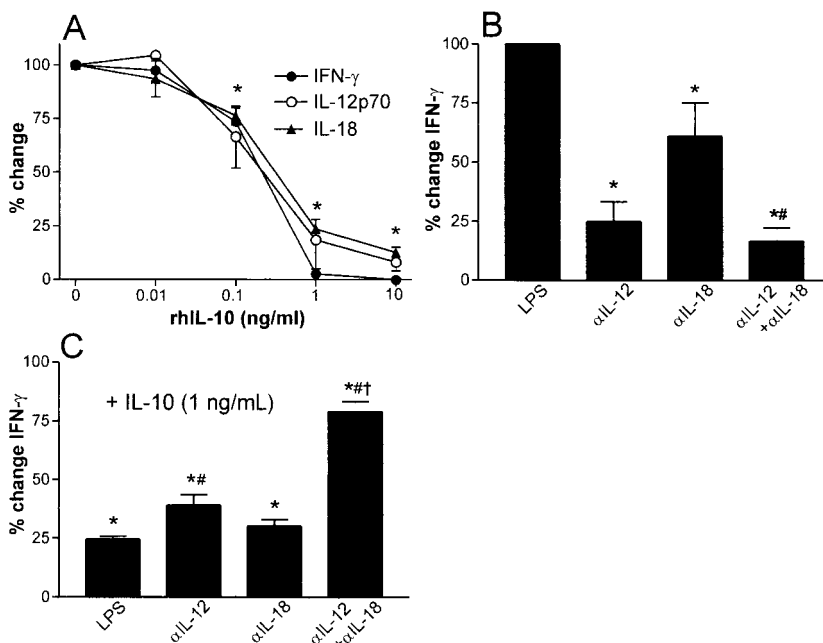


FIGURE 4. IL-10 inhibits IFN- γ release in whole blood in vitro, which is mediated in part by the inhibitory effect of IL-10 on IL-12 and IL-18. Human whole blood was incubated for 24 h at 37°C with LPS in the presence or absence of IL-10, anti-IL-12, and/or anti-IL-18. Data are mean \pm SE of six donors. **A**, IL-10 dose-dependently inhibits the production of IFN- γ , IL-12p70, and IL-18 during whole blood stimulation with LPS (10 ng/ml). Data are expressed relative to incubation with LPS only (=100%). *, $p < 0.05$ vs incubation with LPS only. **B**, Addition of an anti-IL-12 mAb and/or an anti-IL-18 mAb (both 10 μ g/ml) reduces LPS-induced IFN- γ production. Data are expressed relative to incubation with LPS only (=100%). *, $p < 0.05$ vs incubation with LPS only. #, $p < 0.05$ vs LPS + anti-IL-12 and vs LPS + anti-IL-18. **C**, IL-10 (1 ng/ml) inhibits LPS-induced IFN- γ production in the presence or absence of anti-IL-12 and/or anti-IL-18. Data are expressed relative to incubation without IL-10 (=100%) (i.e., LPS + IL-10 relative to LPS alone; LPS + anti-IL-12 and/or anti-IL-18 + IL-10 relative to LPS + anti-IL-12 and/or anti-IL-18). *, $p < 0.05$ vs incubation without IL-10. #, $p < 0.05$ vs incubation with LPS + IL-10. †, $p < 0.05$ vs LPS + anti-IL-12 + IL-10.

not specific for LPS-induced IFN- γ production because IL-10 also inhibited anti-CD3/anti-CD28, SEB-, or PHA-stimulated IFN- γ release (data not shown).

Discussion

Although IL-10 is considered a potent antiinflammatory cytokine, recent studies have suggested that IL-10 also possesses immunostimulatory effects. The present study is the first to demonstrate that IL-10 exerts proinflammatory effects in humans *in vivo*. IL-10 treatment, especially when administered 1 h after LPS injection, potentiated LPS-induced IFN- γ release, which was associated with elevated levels of the IFN- γ -dependent chemokines IP-10 and Mig. The stimulatory effect of IL-10 on IFN- γ release was not associated with a detectable increase in IFN- γ -inducing cytokines. In addition, IL-10 treatment enhanced activation of CTL and NK cells after LPS injection, as reflected by elevated levels of soluble GrA and GrB. The stimulatory effect of IL-10 on LPS-induced IFN- γ release could not be reproduced in whole blood *in vitro*, in which IL-10 dose-dependently reduced IFN- γ production at least in part by inhibiting the synthesis of IL-12 and IL-18. These data indicate that high-dose IL-10 therapy in patients with inflammatory disorders can be associated with undesired proinflammatory effects *in vivo*.

IL-10 is a potent inhibitor of the production of proinflammatory cytokines including TNF, IL-1 α , IL-1 β , IL-6, and IL-8 (1, 4, 9, 10). We demonstrate here that IL-10 stimulates the production of the proinflammatory cytokine IFN- γ during human endotoxemia. IFN- γ is mainly produced by CD4⁺ Th1 cells, CD8⁺ T cells, and NK cells (22). IL-12 and IL-18 positively regulate IFN- γ production (23, 24). IL-12 is the most potent inducer of IFN- γ synthesis, whereas IL-18 synergistically enhances the IL-12 effect (25, 31). IL-12 is a heterodimeric cytokine consisting of a p35 and a p40 subunit (23). Upon stimulation, IL-12p40 is found in large excess over the biologically active IL-12p70 heterodimer. In our study, IL-12p70 levels remained undetectable after LPS injection, whereas IL-12p40 concentrations transiently increased. Conceivably, LPS induces a rise in circulating IL-12p70 levels only when higher doses are administered. Indeed, during severe Gram-negative bacteremia in baboons, the plasma concentrations of both IL-12p70 and IL-12p40 increase, albeit the former to a lesser extent (32). In mice, IL-12p40 immunoreactivity detected after LPS injection comprises both p40 monomers and p40 homodimers, the latter comprising 20–40% of the total p40 produced (33). To our knowledge, it has not been demonstrated that human IL-12p40 homodimers can be detected during human endotoxemia. On human T cell lines, IL-12p40 homodimers have been found to inhibit binding of IL-12 without mediating biological activity (34). It has been found that murine IL-12p40 homodimers can function as IL-12 receptor antagonists (33, 35), but may have immunostimulatory effects on CD8⁺ cells, resulting in IFN- γ production (36). Because IL-10 strongly reduced LPS-induced IL-12p40 release, it is not likely that the positive effect of IL-10 on IFN- γ secretion was mediated by an effect on IL-12p40 production. Similarly, because IL-18 concentrations remained unchanged in all subjects, IL-10 effects on IL-18 production likely did not play a role. Other cytokines implicated in IFN- γ production either remained undetectable (IL-15) or were inhibited (TNF) by IL-10 (the present study and Ref. 10). Together, these data suggest that the stimulatory effect of IL-10 on IFN- γ production *in vivo* is mediated by other pathways or a direct effect of IL-10 on IFN- γ producing cells.

Results from our study are in contrast with data found in a previous study in which human volunteers were injected with in-

creasing doses (1, 10, or 25 $\mu\text{g}/\text{kg}$) of rhIL-10 (37, 38). PBMCs, isolated at 3 h and 6 h after IL-10 administration at a dose of 10 or 25 $\mu\text{g}/\text{kg}$, produced less IFN- γ during *in vitro* stimulation with PHA and PMA compared with PBMCs isolated from volunteers receiving placebo (38). However, the inhibitory effect was stronger when IL-10 was administered at a dose of 10 $\mu\text{g}/\text{kg}$, indicating that high-dose IL-10 may not only be associated with antiinflammatory effects. In this study, PHA and PMA were used as stimuli, which leads to T cell stimulation, whereas we used LPS as a stimulus, which targets monocytes/macrophages. Because IFN- γ is produced by T and NK cells, and LPS has no direct effect on these cells, it could be expected that other stimuli could result in a similar stimulating effect. However, it seems likely that priming of T and NK cells, either through direct or indirect stimulation, is needed for an immunostimulatory effect of IL-10 administration *in vivo*.

Previous *in vitro* studies have demonstrated that IL-10 can have a direct stimulatory effect on activated CD8⁺ T cells and NK cells. IL-10 enhances IL-2-stimulated proliferation of purified human CD8⁺ cells (13). Addition of IL-10 to mouse NK cells enhances IFN- γ production when they are stimulated with IL-12 and IL-18 (14). A recent study showed that IL-10 enhanced the capacity of IL-18 to stimulate IFN- γ production, cytotoxicity, and proliferation of murine NK cells (39). Also, in transgenic mice expressing human IL-10, tumor cell growth was importantly inhibited compared with tumor growth in nontransgenic controls, an effect that was mediated in part by IL-10-activated CD8⁺ T cells (40). To determine whether IL-10 treatment during endotoxemia involves increased activation of CD8⁺ T cells and NK cells *in vivo*, we measured concentrations of GrA and GrB. Granzymes are a family of serine proteinases present in cytoplasmic granules of CTL and NK cells (29, 41) that are released upon activation and can trigger pathways of apoptosis in the target cells (29, 42). Measurements of levels of soluble granzymes is considered to reflect the involvement of CTL and NK cells in various disease states, and increased plasma levels of soluble granzymes have been found in patients with rheumatoid arthritis, EBV, or HIV-1 infection, and during primary cytomegalovirus infection (21, 43). Levels of GrA and GrB were modestly elevated after LPS administration, but IL-10 treatment induced a significant increase in levels of both GrA and GrB. These data suggest that IL-10 treatment results in enhanced activation of cytotoxic lymphocytes leading to increased IFN- γ production.

The increase in IFN- γ levels after IL-10 posttreatment was accompanied by enhanced release of IP-10 and Mig, both members of the CXC chemokine family. *In vitro*, IP-10 and Mig are produced by a variety of cells in response to IFN- γ (28). IP-10 and Mig are closely related and share a common receptor, CXCR3, which is preferentially expressed on activated Th1 cells. IP-10 and Mig are potent chemoattractants for activated T cells and hereby play an important role in inflammatory processes. We here report that levels of IP-10 and Mig increase during human endotoxemia, indicating that these chemokines may play a role during systemic infection. IP-10 concentrations peaked early, whereas levels of Mig remained elevated until the end of the study period, indicating that these proteins are differentially regulated. Although IL-10 pretreatment had no effect on IP-10 levels and inhibited the early increase of Mig, IL-10 posttreatment enhanced both IP-10 and Mig. Because only IL-10 posttreatment increased IP-10 and Mig levels, whereas IFN- γ was also increased with IL-10 pretreatment, these data suggest that *in vivo*, besides IFN- γ , other factors are involved in IP-10 and Mig production. *In vitro*, TNF has been shown to be a costimulus for IFN- γ -induced IP-10 and Mig production (44). Previously, we have reported that only pretreatment

with IL-10 inhibited TNF production during human endotoxemia (10), suggesting that IL-10 posttreatment was more effective in stimulating IP-10 and Mig production due to enhancement of IFN- γ release in the absence of a concurrent reduction in TNF concentrations.

The IL-10-induced enhancement of IFN- γ release in vivo could not be reproduced during human whole blood stimulation with LPS in vitro. We used whole blood stimulation rather than stimulation of isolated cells because the former system is considered to mimic in vivo conditions best, containing hormones, cytokines, and other soluble factors that may influence cytokine production (30). This method has been adopted by many different groups, and has been used extensively to study the regulation of cytokine production (19, 37, 38, 45–49). In the whole blood experiments, IL-10 induced a strong dose-dependent inhibition of LPS-induced IFN- γ production, which was associated with a concurrent reduction in IL-12 and IL-18 concentrations. These findings confirm and extend a previous study in which IL-10 was reported to attenuate IFN- γ and IL-12 production by LPS-stimulated PBMCs (50). Because we considered it possible that IFN- γ release after low dose LPS administration to humans occurs at least in part independently from IL-12 and IL-18 (i.e., circulating concentrations of IL-12p70 and IL-18 remained undetectable and unchanged respectively), we also evaluated the effect of IL-10 on LPS-induced IFN- γ release in whole blood in vitro in the absence of IL-12 and IL-18 activity. However, in the presence of anti-IL-12 and/or anti-IL-18, IL-10 still inhibited LPS-induced IFN- γ production, although to a lesser extent, indicating that IL-10 inhibits IFN- γ release in vitro in part through inhibition of IFN- γ -inducing cytokines. Hence, the effects found of IL-10 on IFN- γ production in vivo likely involve cell populations or mediators, which are not present or present in low quantities in peripheral blood. Discrepancies between effects of experimental interventions observed in vivo or in vitro have been described previously. Addition of anti-TNF Abs has been reported to have no effect on LPS-induced IL-6 and IL-1 β release during human whole blood stimulation in vitro (45). In contrast, during experimental endotoxemia in chimpanzees, injection of anti-TNF strongly inhibited the production of IL-6 (51). Also, Abs to TNF importantly reduced the release of IL-6 and IL-1 β during experimental bacteremia in baboons (52). Furthermore, we previously reported that incubation with rhIL-10 did not significantly influence LPS-stimulated monocyte chemoattractant protein (MCP)-1 production in whole blood in vitro, whereas administration of rhIL-10 during experimental human endotoxemia strongly attenuated MCP-1 release (53).

IL-10 is evaluated as a new adjuvant therapy for several inflammatory diseases (16, 17). Treatment of Crohn's disease with IL-10 has been found to be associated with a bell-shaped dose-response curve (54). At a dose of 5–10 $\mu\text{g}/\text{kg}$ s.c., IL-10 has been reported to induce clinical remissions, but at higher-dose IL-10 (20 $\mu\text{g}/\text{kg}$) the beneficial effects are lost. In the present study, we demonstrate that IL-10 used at a dose of 25 $\mu\text{g}/\text{kg}$, particularly when administered shortly after an inflammatory stimulus, has proinflammatory effects in humans in vivo. Therefore, these data indicate that the potential IFN- γ -enhancing effect of IL-10 may especially warrant caution for the use of high-dose IL-10 therapy for Th1-mediated illnesses like Crohn's disease and rheumatoid arthritis.

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