Epinephrine Inhibits Tumor Necrosis Factor-α and Potentiates Interleukin 10 Production during Human Endotoxemia

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Abstract

Short-term preexposure of mononuclear cells to epinephrine inhibits LPS-induced production of TNF, whereas preexposure for 24 h results in increased TNF production. To assess the effects of epinephrine infusions of varying duration on in vivo responses to LPS, the following experiments were performed: (*a*) Blood obtained from eight subjects at 4–24 h after the start of a 24-h infusion of epinephrine (30 ng/kg per min) produced less TNF after ex vivo stimulation with LPS compared with blood drawn before the start of the infusion, and (*b*) 17 healthy men who were receiving a continuous infusion of epinephrine (30 ng/kg per min) started either 3 h (EPI-3; *n* = 5) or 24 h (EPI-24; *n* = 6) before LPS injection or an infusion of normal saline (LPS; *n* = 6) were studied after intravenous injection of LPS (2 ng/kg, lot EC-5). EPI-3 inhibited LPS-induced in vivo TNF appearance and also increased IL-10 release (both *P* < 0.005 versus LPS), whereas EPI-24 only attenuated TNF secretion (*P* = 0.05). In separate in vitro experiments in whole blood, epinephrine increased LPS-induced IL-10 release by a combined effect on α and β adrenergic receptors. Further, in LPS-stimulated blood, the increase in IL-10 levels caused by epinephrine only marginally contributed to concurrent inhibition of TNF production. Epinephrine, either endogenously produced or administered as a component of sepsis treatment, may have a net antiinflammatory effect on the cytokine network early in the course of systemic infection. (*J. Clin. Invest.* 1996. 97:713–719.) Key words: lipopolysaccharide • cytokines • adrenergic receptors • norepinephrine • cAMP

Introduction

The systemic inflammatory response syndrome associated with sepsis involves activation of both the immune and the neuroendocrine system. Sepsis leads to excessive production of proinflammatory cytokines, which is considered to contribute to the development of organ failure and tissue injury (1, 2). Administration of TNF or IL-1 to humans or animals reproduces the major features of systemic infection, including a stress hormone response (3–5). Additionally, stress hormone release during the early phases of a septic insult is mediated at least in part by the action of proinflammatory cytokines (6–8).

In recent years it has become clear that complex interactions exist between stress hormones and the cytokine network. The catecholamines epinephrine and norepinephrine have been shown to inhibit the production of TNF by mononuclear cells and in human whole blood stimulated with endotoxin (LPS) (9, 10). In accordance, pretreatment with epinephrine was associated with an attenuated TNF response after administration of LPS to mice (11). Interestingly, whereas preexposure of mononuclear cells to epinephrine for 3 h strongly inhibited TNF production elicited by subsequent incubation with LPS, preexposure to epinephrine for 24 h was associated with enhanced TNF synthesis, suggesting that the influence of epinephrine on cytokine release is determined partially by the duration of inflammatory cell exposure to catecholamines (9).

The in vivo effects of epinephrine on the cytokine network during an acute inflammatory response in humans are unknown. Knowledge of such effects may have implications not only for the understanding of endogenous stress hormone influences during injury, but also for the therapeutic use of catecholamines in patients with septic shock. Therefore, in the present study we sought to determine the effect of a constant epinephrine infusion, started either 3 or 24 h before intravenous injection of endotoxin, on the induction of the cytokine network.

Methods

Study design and subjects. 19 male subjects, aged 28±1 (mean±SE) yr, were admitted to the Adult Clinical Research Center of the New York Hospital–Cornell University Medical Center after documentation of good health by history, physical examination, and hematologic and biochemical screening. The study was approved by the Institutional Review Board, and written informed consent was obtained from all subjects before enrollment in the study. The volunteers were admitted for 4 d (day 0–day 3). On day 1, subjects were randomized to receive either a constant intravenous infusion of epinephrine (Parke-Davis, Morris Plains, NJ; 30 ng/kg per min; *n* = 8), starting at 9:00 a.m. or an equivalent volume of normal saline (*n* = 11). On day 2, at 9:00 a.m., six of eight subjects who were started on epinephrine the previous day were intravenously injected with a single dose of endotoxin (National Reference Endotoxin, *Escherichia coli* 0113, lot EC-5, generously provided by Dr. H. D. Hochstein, the Bureau of Biologics, Food and Drug Administration, Bethesda, MD) at a dose of 2 ng/kg body wt (EPI-24 group). The infusion of epinephrine was continued until 6 h after LPS administration (3:00 p.m.). The 11 subjects who received only normal saline on day 1 were randomized on day 2 to receive either a constant intravenous infusion of epinephrine (30 ng/kg per min; *n* = 5), starting at 6:00 a.m. (3 h before endotoxin administration) and continued until 3:00 p.m. (EPI-3 group), or an equivalent volume of normal saline (*n* = 6) (LPS group). These subjects were also injected with endotoxin (2 ng/kg) at 9:00 a.m. Epinephrine was freshly prepared in normal saline every 8 h. The final

epinephrine solution contained ascorbic acid (Cenolate; Abbott Laboratories, North Chicago, IL; 1 mg/ml). Ascorbic acid at concentrations from 1 to 100 μg/ml did not influence LPS-induced cytokine production in whole blood in vitro (data not shown). 3 h before the administration of endotoxin, a radial arterial catheter was placed in all subjects to monitor heart rate and blood pressure continuously (model 2000A; Datascope Corp., Paramus, NJ) and for blood sampling. A rectal probe was inserted to allow continuous measurement of core temperature. On day 1 all subjects were given a defined formula oral diet (Sustacal; Mead-Johnson & Co., Evansville, IN) in four equal portions (total 30 kcal/kg). From day 1, 10:00 p.m., until day 2, 9:00 p.m., all volunteers were fasted.

On day 1, venous blood was obtained before the start of infusion with epinephrine or normal saline ($t = 0$ h), and at 1, 2, 4, and 8 h thereafter. On day 2, arterial blood was obtained at 6:00 a.m. (i.e., directly before the start of the infusion of epinephrine in the EPI-3 group or the start of saline infusion in the control group) ($t = -3$ h), directly before the injection of endotoxin ($t = 0$ h), and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, and 24 h thereafter. All blood samples (except samples for leukocyte counts) were centrifuged at 4°C for 20 min at 1,600 g and stored at −70°C until assayed.

Whole blood stimulation. On day 1 (0, 4, and 8 h) and day 2 (−3 and 0 h), blood was drawn to assess the effect of various durations of epinephrine infusion on endotoxin responsiveness in human whole blood. Blood was collected aseptically using a sterile collecting system consisting of a butterfly needle connected to a syringe (Becton Dickinson & Co., Rutherford, NJ). Anticoagulation was obtained using sterile heparin (Elkins-Sinn Inc., Cherry Hill, NJ) (10 U/ml blood, final concentration). Heparin was chosen as anticoagulant rather than EDTA in whole blood experiments, since EDTA has been reported to inhibit cell function in bioassays and to inhibit the production of TNF (12). Incubation of heparinized whole blood in the absence of LPS did not result in detectable cytokine production. Whole blood was stimulated for 24 h at 37°C with LPS (10 ng/ml, final concentration; *E. coli* serotype 0127:B8; Sigma Chemical Co, St. Louis, MO) in sterile polypropylene tubes (Becton Dickinson & Co.) as described previously (10, 13). After the incubation, plasma was prepared by centrifugation and stored at −70°C until assays were performed. TNF levels were expressed as nanograms per 10^9 monocytes, since monocyte counts changed during infusion of epinephrine and monocytes are the major source of TNF (14).

In separate in vitro experiments, whole blood was diluted 1:1 in sterile RPMI 1640 supplemented with L-glutamine (GIBCO BRL, Life Technologies Inc., Grand Island, NY). In these experiments, LPS (10 ng/ml) was incubated for various time periods (4–24 h) in the presence or absence of the following adrenergic agonists and antagonists: epinephrine (Parke-Davis), phentolamine (Ciba-Geigy, Basel, Switzerland), propranolol (Ayerst, Philadelphia, PA), norepinephrine (Abbott Laboratories), phenylephrine (American Regent Laboratories, Shirley, NY), isoproterenol (Sanofi Winthrop Pharmaceuticals, New York), terbutaline (Ciba Geigy), and UK-14,304 (kindly provided by Pfizer Ltd., Sandwich, UK). In addition, in some whole blood stimulations, neutralizing mAbs directed against human TNF (3C3; Medgenix, Fleurus, Belgium), or human IL-10 (IF9; Medgenix) were used. Anti–human-FSH mAb was used as an isotype-matched control antibody in these experiments.

Assays. TNF (CLB, Amsterdam, The Netherlands) (13), IL-1β (15), IL-6 (16), IL-8 (17), IL-10 (Pharmingen, San Diego, CA) (18), and IL-1 receptor antagonist (R&D Systems, Minneapolis, MN) were measured in heparinized plasma using specific ELISAs. The reagents for the IL-1β ELISA were kindly provided by Dr. John S. Kenney. Leukocyte counts and differentials were determined in K_2-EDTA anticoagulated blood using flow cytometry. Blood for measurement of epinephrine was collected in tubes preloaded with ethyleneglycol-tetraacetic acid and reduced glutathione (Amersham Corp., Arlington Heights, IL). Plasma concentrations of epinephrine were measured with a radioenzymatic assay (Amersham Corp.). Cortisol was measured by radioimmunoassay (19).

Statistical analysis. All values are given as means±SEM. Serial data in normal subjects were analyzed by analysis of variance. Two sample comparisons were performed using the Wilcoxon test. $P < 0.05$ was considered to represent a statistically significant difference.

Results

Endotoxin-induced TNF production by whole blood ex vivo during epinephrine infusion (day 1). During infusion of saline, plasma epinephrine concentrations and monocyte counts did not change and remained normal. In addition, LPS-induced TNF production by whole blood was similar at all time points evaluated, indicating that there was no circadian rhythm that influenced LPS responsiveness of whole blood (data not shown). In subjects receiving a constant infusion of epinephrine, plasma epinephrine concentrations reached a plateau of 1,037±179 pg/ml, whereas monocyte counts modestly increased (data not shown). Epinephrine significantly attenuated LPS-induced TNF production in whole blood (Fig. 1). Inhibition of TNF synthesis was noted with three different concentrations of in vitro LPS stimulation (1, 10, and 100 ng/ml; all $P < 0.0001$ versus $t = 0$). Further, this effect was noted within 4 h after initiating epinephrine infusion and persisted throughout the 24-h observation period (Fig. 1).

Effect of epinephrine on endotoxin-induced cytokine production in vivo (day 2). LPS administration was associated with a modest rise in epinephrine levels, peaking after 2 h (from 108±53 pg/ml at baseline to 231±56 pg/ml; $P < 0.05$). In subjects infused with epinephrine, plasma levels of epinephrine did not change after LPS administration, plateau concentrations 1,111±224 pg/ml in the EPI-3 group and 1,213±246 pg/ml in the EPI-24 group ($P = 0.34$ for the difference between groups). Administration of LPS elicited comparable influenza-like symptoms, such as headache, chills, and muscle aches, in all treatment groups. LPS injection elicited a rise in body temperature, peaking after 3–4 h (38.2±0.2°C; $P < 0.0001$ versus baseline), which was not influenced by EPI-3 or EPI-24 (data not shown).
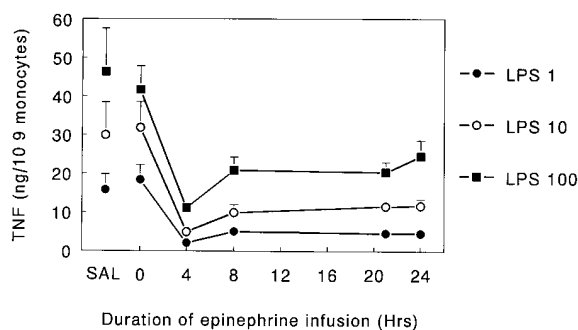

Figure 1. Plasma concentrations of TNF (ng/10^9 monocytes) after stimulation of whole blood, obtained during a constant intravenous infusion of epinephrine, with LPS. Blood was drawn directly before ($t = 0$) and during a constant intravenous infusion of epinephrine (30 ng/kg per min) at $t = 4, 8, 21$, and 24 h. Whole blood was then incubated with 1, 10, or 100 ng/ml LPS for 24 h at 37°C, after which plasma was collected. Data are mean (±SE) of eight normal subjects. *SAL*, plasma TNF levels after LPS stimulation of whole blood obtained at $t = 0$ h from 11 other subjects who were subsequently infused with saline for 24 h. In saline-infused subjects, LPS-induced TNF production by whole blood was similar at all time points after the start of saline infusion (not shown).
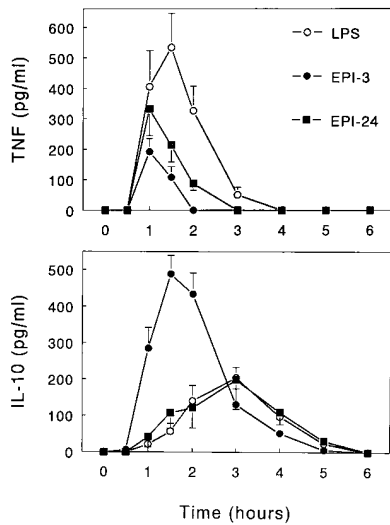
Figure 2. Mean (±SE) plasma concentrations of TNF (*top*) and IL-10 (*bottom*) after intravenous injection of LPS (2 ng/kg, lot EC-5) at $t = 0$. *LPS*, subjects injected with LPS only ($n = 6$); *EPI-3*, subjects infused with epinephrine (30 ng/kg per min) from $t = -3$ to 6 h ($n = 5$); *EPI-24*, subjects infused with epinephrine (30 ng/kg per min) from $t = -24$ to 6 h ($n = 6$). EPI-3 attenuated the LPS-induced increase in TNF levels and potentiated the rise in IL-10 levels (both $P < 0.0005$ versus LPS only). EPI-24 only reduced TNF levels ($P = 0.05$ versus LPS only), without an effect on IL-10 release.
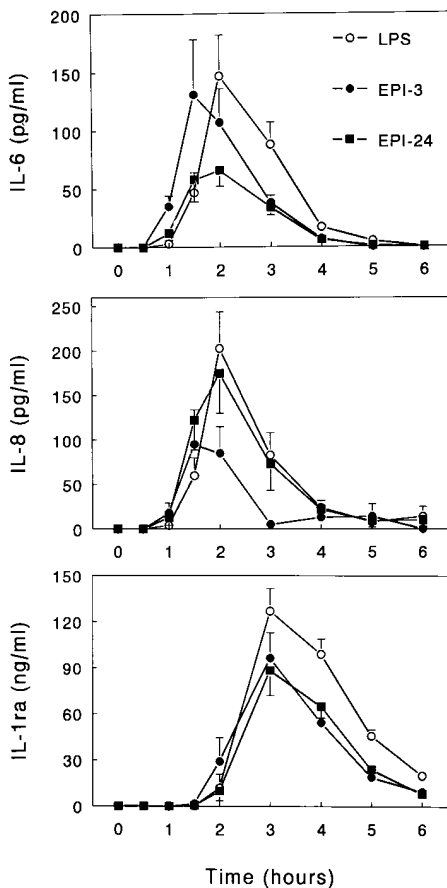

Figure 3. Mean (±SE) plasma concentrations of IL-6, IL-8, and IL-1 receptor antagonist after intravenous injection of LPS (2 ng/kg, lot EC-5) at $t = 0$. *LPS*, subjects injected with LPS only ($n = 6$); *EPI-3*, subjects infused with epinephrine (30 ng/kg per min) from $t = -3$ to 6 h ($n = 5$); *EPI-24*, subjects infused with epinephrine (30 ng/kg per min) from $t = -24$ to 6 h ($n = 6$). There were no significant differences between the increases in IL-6, IL-8, and IL-1 receptor antagonist in the three treatment groups.

On day 1 during infusion of epinephrine (without injection of LPS), TNF, IL-1β, IL-6, and IL-8 remained undetectable in plasma, whereas IL-1 receptor antagonist levels remained unaltered (data not shown). On day 2, injection of LPS only induced a transient rise in plasma TNF concentrations, peaking after 1.5 h (535±111 pg/ml; $P < 0.0001$ versus baseline; Fig. 2). Both EPI-3 and EPI-24 attenuated LPS-induced TNF release ($P < 0.005$ and $P = 0.05$ versus LPS only, respectively); EPI-3 had the more pronounced effect (Fig. 2, *top*). Peak TNF concentrations were 192±44 pg/ml in the EPI-3 group and 333±88 pg/ml in the EPI-24 group. It is also of interest that peak TNF levels in both groups treated with epinephrine occurred slightly earlier (after 1 h) than after injection of LPS only (1.5 h). LPS also elicited a monophasic rise in the plasma concentrations of IL-10 (3 h, 205±28 pg/ml; $P < 0.0001$). EPI-3 strongly potentiated LPS-induced IL-10 release ($P < 0.005$ versus LPS only), which was also associated with a shift of the peak IL-10 response to earlier time points (1.5 h, 489±51 pg/ml; Fig. 2, *bottom*). By contrast, EPI-24 influenced neither the extent nor the time course of LPS-induced IL-10 release. In this treatment group, peak IL-10 levels were achieved after 3 h (199±81 pg/ml). Epinephrine also tended to reduce LPS-induced release of IL-6, IL-8, and IL-1 receptor antagonist, but these effects did not reach statistical significance (Fig. 3). IL-1β remained undetectable in all groups at all time points.

Epinephrine did not influence the increase in plasma cortisol concentrations induced by intravenous endotoxin. Peak levels were reached after 3 h in all treatment groups (LPS only, 301±14 ng/ml, EPI-3, 303±32 ng/ml; EPI-24, 290±24 ng/ml).

Contribution of α and β adrenergic receptors to potentiation of LPS-induced IL-10 production by epinephrine. To further investigate the mechanism by which epinephrine potentiated LPS-induced IL-10 production, we next performed a number of in vitro experiments on human whole blood. Unless stated otherwise, whole blood was incubated with 10 ng/ml LPS at 37°C for 16 h, after which supernatant plasma was collected for TNF and IL-10 measurement. A 16-h incubation was chosen because preliminary experiments had established that IL-10 levels were maximal in LPS-stimulated blood at that time point. Epinephrine dose dependently inhibited LPS-induced TNF production, maximal inhibition achieved at a concentration of 10^{-6} M (Fig. 4). In addition, epinephrine caused a dose-
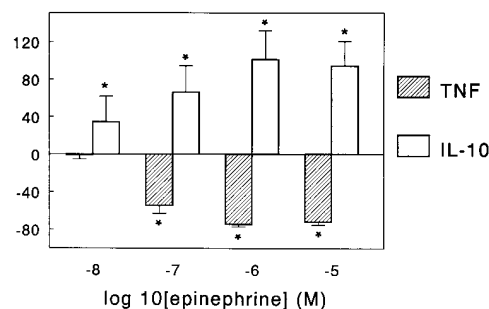

Figure 4. Epinephrine dose dependently inhibits TNF production and potentiates IL-10 production in LPS-stimulated whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 16 h with LPS (10 ng/ml) in the presence or absence of increasing concentrations of epinephrine. Cytokine levels are expressed as percent change relative to levels measured after incubation with LPS only. Data are mean±SE of eight different donors. *$P < 0.05$ versus LPS only.
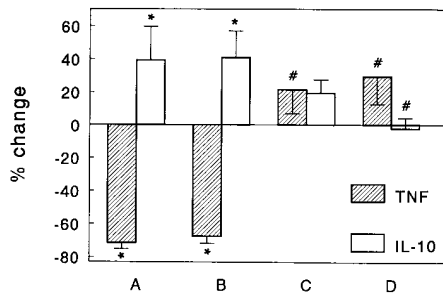
Figure 5. Effect of α and/or β receptor blockade on the effect of epinephrine on TNF and IL-10 production by LPS-stimulated whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 16 h with LPS (10 ng/ml). *A*, with epinephrine (10^{-6} M); *B*, with epinephrine (10^{-6} M) and the α_1 and α_2 antagonist phentolamine (10^{-5} M); *C*, with epinephrine (10^{-6} M) and the β_1 and β_2 antagonist propranolol (10^{-5} M); *D*, with epinephrine, phentolamine, and propranolol. Cytokine levels are expressed as percent change relative to cytokine levels measured after incubation with LPS only. Data are mean±SE of six different donors. *$P < 0.05$ versus LPS only. #$P < 0.05$ versus LPS with epinephrine.

dependent enhancement of LPS-induced IL-10 production, an effect that was also maximal at a concentration of 10^{-6} M (Fig. 4). The extent and direction of the effect of epinephrine on TNF and IL-10 production were similar after various durations of incubations of whole blood with LPS (4, 8, 12, 16, or 24 h) (data not shown).

Epinephrine binds to both α (α_1 and α_2) and β (β_1 and β_2) adrenergic receptors (20). We next assessed which adrenergic receptor was involved in the effects of epinephrine on IL-10 production. For this purpose, we incubated whole blood with LPS (10 ng/ml) in the presence or absence of epinephrine (10^{-6} M), the α (α_1 and α_2) adrenergic receptor antagonist phentolamine (10^{-5} M), and/or the β (β_1 and β_2) receptor antagonist propranolol (10^{-5} M). As previously established (9), propranolol completely restored LPS-induced TNF production in the presence of epinephrine (Fig. 5). Blockade of α receptors by phentolamine did not influence the epinephrine in-
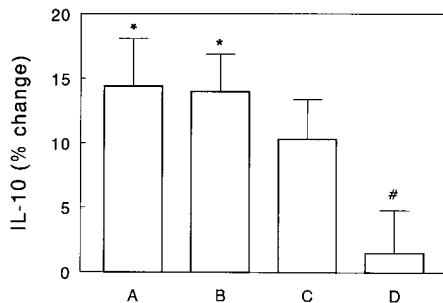

Figure 6. Effect of α and/or β receptor blockade on the effect of norepinephrine on IL-10 production by LPS-stimulated whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 16 h with LPS (10 ng/ml). *A*, with norepinephrine (10^{-6} M); *B*, with norepinephrine (10^{-6} M) and the α_1 and α_2 antagonist phentolamine (10^{-5} M); *C*, with norepinephrine (10^{-6} M) and the β_1 and β_2 antagonist propranolol (10^{-5} M); *D*, with norepinephrine, phentolamine, and propranolol. IL-10 levels are expressed as percentage change relative to IL-10 levels measured after incubation with LPS only. Data are mean±SE of six different donors. *$P < 0.05$ versus LPS only. #$P < 0.05$ versus LPS with norepinephrine.
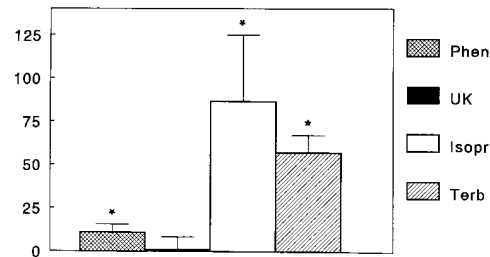

Figure 7. Effect of specific α or β receptor stimulation on LPS-induced IL-10 production by whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 16 h with LPS (10 ng/ml) with α or β agonists (10^{-7} M). *Phen*, with phenylephrine (α agonist with predominant affinity for the α_1 receptor); *UK*, UK-14,304 (a specific α_2 agonist); *Isopr*, isoproterenol ($\beta_1 + \beta_2$ agonist); *Terb*, terbutaline (β receptor agonist with predominant affinity for the β_2 receptor). IL-10 levels are expressed as percent change relative to IL-10 levels measured after incubation with LPS only. Data are mean±SE of six different donors. *$P < 0.05$ versus LPS only.

hibition of TNF production. By contrast, propranolol only partially influenced the effect of epinephrine on LPS-induced IL-10 production (nonsignificant), whereas the combination of phentolamine and propranolol completely prevented the epinephrine effect on IL-10 production (Fig. 5). To confirm that both α and β receptor stimulation are involved in upregulation of LPS-induced IL-10 production, we next incubated norepinephrine (an $\alpha_1 + \alpha_2 + \beta_1$ agonist) at 10^{-6} M with LPS in the presence or absence of phentolamine (10^{-5} M) and/or propranolol (10^{-5} M). As shown in Fig. 6, norepinephrine modestly, but significantly, potentiated LPS-induced IL-10 production, an effect that could only be reversed by simultaneous α and β receptor blockade.

We then sought to determine whether specific α and β receptor stimulation could mimic the effect of epinephrine on IL-10 production. Whole blood was incubated with LPS and specific α or β adrenergic agonists. As depicted in Fig. 7, isoproterenol ($\beta_1 + \beta_2$ receptor agonist) and terbutaline (β receptor agonist with predominant affinity for the β_2 receptor) were potent stimulators of LPS-induced IL-10 release, whereas phenylephrine (an α receptor agonist with predominant affinity for the α_1 receptor) modestly enhanced IL-10 secretion in LPS-stimulated whole blood. By contrast, a specific α_2 receptor agonist (UK-14,304) did not influence IL-10 levels.

Potentiation of IL-10 production marginally contributes to the inhibiting effect of LPS-induced TNF production by epinephrine. IL-10 is known to inhibit LPS-induced TNF production in vitro and in vivo (21–24). It was therefore possible that epinephrine inhibits LPS-induced TNF production in whole blood at least in part by enhancing the release of IL-10. To test this hypothesis, we incubated whole blood for various time periods (4, 8, 12, and 16 h) with LPS (10 ng/ml) in the presence or absence of epinephrine (10^{-6} M), a neutralizing anti–IL-10 mAb (25 μg/ml) or an equivalent amount of an irrelevant isotype-matched control mAb. Anti–IL-10 potentiated LPS-induced TNF production, an effect that became significant after incubations of whole blood for 8 h or longer (Table I). In the presence of anti–IL-10, epinephrine continued to inhibit LPS-induced TNF production, although the extent of TNF inhibition was less after incubations of 8, 12, and 16 h (10% less), than in the absence of anti–IL-10 (Fig. 8).

Incubation	TNF			
	LPS	LPS + EPI	LPS + anti–IL-10	LPS + EPI + anti–IL-10
h	*ng/ml*			
4	4.74±0.49	1.48±0.23*‡	4.72±0.40	1.53±0.23*‡
8	6.61±0.71	1.73±0.29*‡	8.40±0.73*	3.00±0.50*‡
12	5.64±0.53	1.40±0.20*‡	7.96±0.77*	2.84±0.41*‡
16	4.75±0.50	1.19±0.18*‡	7.27±0.96*	2.58±0.44*‡

Whole blood diluted 1:1 in RPMI 1640 was incubated for 4–16 h with LPS (10 ng/ml) in the presence or absence of epinephrine (EPI, 10^{-6} M), anti–IL-10 mAb (25 μg/ml), and/or an irrelevant control mAb (25 μg/ml). TNF levels are mean±SE of six different donors. *$P < 0.05$ versus LPS only. ‡$P < 0.05$ versus LPS + anti–IL-10.

Incubation	IL-10			
	LPS	LPS + EPI	LPS + anti-TNF	LPS + EPI + anti-TNF
h	*ng/ml*			
8	0.30±0.09	0.44±0.12*	0.30±0.09	0.47±0.10‡
16	0.99±0.32	1.45±0.44*‡	0.72±0.21*	1.13±0.30‡
24	0.83±0.24	1.43±0.38*‡	0.66±0.18*	1.25±0.33‡

Whole blood diluted 1:1 in RPMI 1640 was incubated for 8–24 h with LPS (10 ng/ml) in the presence or absence of epinephrine (EPI, 10^{-6} M), anti-TNF mAb (25 μg/ml), and/or an irrelevant control mAb (25 μg/ml). IL-10 levels are mean±SE of five different donors. *$P < 0.05$ versus LPS only. ‡$P < 0.05$ versus LPS + anti-TNF.

Inhibition of TNF production partially masks the potentiation of LPS-induced IL-10 production by epinephrine. It has been demonstrated that the production of IL-10 during endotoxemia in vivo is partly dependent on TNF production (18). Inhibition of TNF production by epinephrine could therefore mask an even more pronounced potentiating effect on IL-10 production by epinephrine. To evaluate this possibility, we incubated whole blood for 8, 16, or 24 h with LPS (10 ng/ml), in the presence or absence of epinephrine (10^{-6} M), a neutralizing anti-TNF mAb (25 μg/ml), or an equivalent amount of an irrelevant isotype-matched control mAb (Table II). Anti-TNF inhibited LPS-induced IL-10 production after incubations of 16 h and longer, indicating that TNF is, indeed, partially responsible for LPS-induced IL-10 production in whole blood. Further, in the presence of anti-TNF, epinephrine caused a significantly more pronounced enhancement of IL-10 production after incubations of 16 and 24 h (20–25% more) than in the abs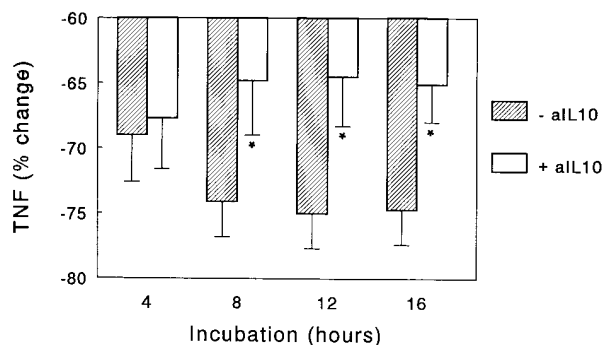ence of anti-TNF (Fig. 9). These data suggested that the (enhancing) effect of epinephrine on IL-10 production is partially reduced by the concurrent (inhibiting) effect of epinephrine on TNF production.

Discussion

Previous research has established that stimulation of β adrenergic receptors inhibits LPS-induced TNF production by mononuclear cells and whole blood in vitro (9–11, 25, 26). Furthermore, epinephrine and the β receptor agonist salmeterol have been reported to attenuate the release of TNF after administration of LPS to mice (11, 26), and epinephrine inhibited LPS-induced TNF production in an isolated perfused rat liver model (27). The present study is the first to examine the effect of epinephrine on cytokine production in humans in vivo. The dose of epinephrine administered was chosen to mimic two clinically relevant situations. First, epinephrine levels achieved were in the same range as those reported in patients with septic shock (6). Second, the rate and dose at which epinephrine
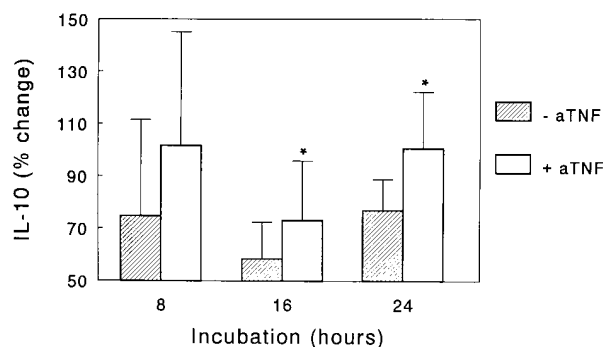
Figure 8. Potentiation of IL-10 production contributes to the inhibiting effect of epinephrine on TNF production in LPS-stimulated whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 4, 8, 12, or 16 h with LPS (10 ng/ml) in the presence or absence of epinephrine (10^{-6} M) and in the presence or absence of anti–IL-10 mAb (25 μg/ml). TNF levels are expressed as percent change relative to TNF levels measured after incubation without epinephrine (i.e., LPS with epinephrine relative to LPS alone; LPS with epinephrine and anti–IL-10 relative to LPS with anti–IL-10). Data are mean±SE of six different donors. *$P < 0.05$ versus no anti–IL-10 mAb.

Figure 9. Inhibition of TNF production partially masks the potentiation of IL-10 production by epinephrine in LPS-stimulated whole blood. Whole blood diluted 1:1 in RPMI 1640 was incubated for 8, 16, or 24 h with LPS (10 ng/ml) in the presence or absence of epinephrine (10^{-6} M) and in the presence or absence of anti-TNF mAb (25 μg/ml). IL-10 levels are expressed as percent change relative to IL-10 levels measured after incubation without epinephrine (i.e., LPS with epinephrine relative to LPS alone; LPS with epinephrine and anti-TNF relative to LPS with anti-TNF). Data are mean±SE of five different donors. *$P < 0.05$ versus no anti-TNF mAb.

was infused were in the same range as the rate and dose at which this catecholamine is started as part of the treatment of patients with sepsis (28).

The inhibition of TNF production by β adrenergic agents has been linked to their stimulating effect on adenylyl cyclase, leading to an increase in intracellular cAMP levels (9, 29–32). The effect of β adrenergic stimulation on cAMP concentrations is transient: While incubation of mononuclear cells with epinephrine or the specific β agonist isoproterenol for 2 h increased intracellular cAMP concentrations, incubation for 24 h was associated with a decrease in cAMP levels (9). This biphasic change in cAMP levels was paralleled by a biphasic effect on LPS-induced production of TNF; i.e., preexposure of mononuclear cells to epinephrine for 3 h inhibited subsequent TNF synthesis, whereas preincubation with epinephrine for 24 h potentiated TNF synthesis (9). By contrast, in the present study, infusion of epinephrine into healthy volunteers for up to 24 h was associated with an inhibition of LPS-induced TNF production, both by whole blood ex vivo and by humans in vivo, although in both settings LPS-induced TNF production was influenced more by shorter preexposure to epinephrine. However, it should be noted that our study was limited in duration and dosage range of the epinephrine infusion and therefore does not exclude the existence of a biphasic effect of epinephrine on LPS-induced TNF production in vivo. In addition, our data do not establish whether epinephrine influences LPS-induced cytokine production in humans in vivo in a dose dependent way, although our in vitro data obtained by LPS stimulation of whole blood suggest that such a dose-dependent effect does exist.

IL-10 inhibits LPS-induced production of TNF by mononuclear cells in vitro and in mice in vivo (21–24) and reduces lethality of endotoxemic mice (23, 24). Production of IL-10 can therefore be considered to be part of a host-protective mechanism during endotoxemia. Subjects who were started on intravenous epinephrine 3 h before the injection of LPS showed a more than doubling of IL-10 release compared with subjects who received LPS only. In whole blood in vitro, stimulation of both α and β adrenergic receptors contributed to the enhancement of LPS-induced IL-10 production by epinephrine. It seems likely that β receptor stimulation contributes more to the effect of epinephrine on IL-10 production. Indeed, mere α receptor blockade had no effect on the upregulation of IL-10 production by epinephrine (or norepinephrine), whereas solely β receptor blockade tended to reduce increased IL-10 levels in the presence of epinephrine or norepinephrine. Furthermore, specific β receptor stimulation with isoproterenol or terbutaline was more potent in increasing LPS-induced IL-10 production than specific α receptor stimulation by phenylephrine. In addition, our results suggest that stimulation of either the β_1 or β_2 receptor can upregulate LPS-induced IL-10 production, since terbutaline (a predominant β_2 agonist), as well as norepinephrine in the presence of an excess of the aspecific α antagonist phentolamine (allowing norepinephrine only to interact with β_1 receptors), were able to enhance IL-10 production. The modest effect of α receptor stimulation on IL-10 synthesis seems to be mediated by the α_1 receptor, since phenylephrine (a predominant α_1 agonist), but not UK-14,304 (a specific α_2 agonist), could produce this effect. Presumably, the enhancement of IL-10 production by β receptor stimulation involves adenylate cyclase, since agents that are known to increase intracellular cAMP levels augment LPS-induced IL-10

synthesis by murine peritoneal macrophages and human whole blood (33, 34).

Since recombinant IL-10 inhibits LPS-induced TNF production (21–24), and neutralization of endogenous IL-10 potentiates TNF production in endotoxemic mice (35), we hypothesized that part of the epinephrine-induced inhibition of TNF release was caused by enhancement of IL-10 release. Therefore, to eliminate the effect of increased IL-10 levels in the presence of epinephrine, experiments with a neutralizing anti–IL-10 mAb were performed. Anti–IL-10 enhanced LPS-induced TNF production in whole blood, confirming a previous report (36). In the presence of anti–IL-10, epinephrine continued to inhibit LPS-induced TNF production by whole blood, albeit to a slightly lesser extent. In other experiments, addition of a neutralizing anti-TNF mAb to whole blood resulted in a reduction of LPS-induced IL-10 release, indicating that TNF contributes to IL-10 production in this system. Therefore, inhibition of TNF release by epinephrine theoretically could mask an even more pronounced enhancing effect of this catecholamine on IL-10 production. Our experiments provided positive evidence for this hypothesis. Indeed, as shown in Fig. 9, epinephrine enhanced LPS-induced IL-10 release more in the presence of anti-TNF (expressed as percent increase relative to IL-10 levels measured after incubation without epinephrine and in the presence of anti-TNF) than in the absence of anti-TNF (expressed as percent increase relative to IL-10 levels measured after stimulation without epinephrine and in the absence of anti-TNF). Hence, in LPS-stimulated blood, upregulation of IL-10 contributes only marginally to the concurrently observed attenuation of TNF production, and, conversely, the inhibition of TNF release by epinephrine conceals an even stronger stimulation of IL-10 release by this hormone.

Knowledge of the effects of epinephrine on cytokine production may be important for the understanding of neuroendocrine and immune interactions during an ongoing systemic inflammatory response syndrome. The early host response to systemic infection is characterized by activation of the cytokine network and release of stress hormones. Moreover, administration of epinephrine frequently is part of the treatment of patients with septic shock. We demonstrate here that short-term preexposure of healthy humans to a constant infusion of epinephrine before injection of low dose endotoxin attenuates the production of the proinflammatory cytokine TNF and simultaneously potentiates the production of the antiinflammatory cytokine IL-10. Long-term preexposure of subjects to epinephrine had a less pronounced antiinflammatory effect, only reducing endotoxin-induced TNF release. Hence, epinephrine, either endogenously produced or given as a component of sepsis treatment, may act to dampen excessive proinflammatory effects of the cytokine network during the early phases of systemic infection.

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