Catecholamines increase monocyte TNF receptors and inhibit TNF through β2-adrenoreceptor activation

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Guirao, Xavier, Ashwini Kumar, Joshua Katz, Michelle Smith, Edward Lin, Chris Keogh, Steve E. Calvano, and Stephen F. Lowry. Catecholamines increase monocyte TNF receptors and inhibit TNF through β2-adrenoreceptor activation. Am. J. Physiol. 273 (Endocrinol. Metab. 36): E1203–E1208, 1997.—Postinjury deficits in monocyte tumor necrosis factor receptors (moTNFR) activity may alter beneficial functions during an inflammatory response. Several counter-regulatory hormones elicited during inflammation may modulate tumor necrosis factor (TNF) activity, but little is known about their influence on moTNFR. Also, catecholamines inhibit TNF production, but the adrenoreceptor mechanism of this effect has not been fully clarified. To determine the effect of catecholamines and corticosteroids on moTNFR, whole blood was incubated for up to 8 (moTNFR) or 24 h (cytokines) in the presence of lipopolysaccharide (100 ng/ml) and 1) epinephrine (Epi, 10⁻⁶ M), dexamethasone (Dex, 10⁻⁶ M) or both (EpiDex, 10⁻⁶ M) to assess the expression of total moTNFR, moTNFR-I, and moTNFR-II. 2) Epi and norepinephrine (EpiNE, 10⁻⁶ M) and the α₃,β₂-, α₁,β₂-, β₂-, and β₂-adrenergic antagonists were used to assess the role of such adrenoreceptors on total moTNFR and TNF production, and N⁶,²-O-dibutyryl adenosine 3',5'-cyclic monophosphate (DBcAMP) alone or in combination with the phosphodiesterase inhibitor Ro-20–1724/000, to study the cAMP-dependent pathway on total moTNFR. We found that Epi upregulated total moTNFR and moTNFR-II. Dex did not significantly influence total moTNFR or moTNFR-II. Also, EpiNE increased total moTNFR and inhibited TNF by a β₂-dependent mechanism. DBcAMP (10⁻⁵ M) modestly enhanced total moTNFR. This suggests a common mechanism for acutely enhancing moTNFR and attenuation of soluble TNF appearance during conditions of severe stress.

epinephrine; dexamethasone; adenosine 3',5'-cyclic monophosphate; cytokines; lipopolysaccharide; tumor necrosis factor

DESPITE EVIDENCE that an overwhelming inflammatory response to sepsis (29) or severe trauma (21) may induce organ damage, the pattern of organ failure later in the course of systemic inflammatory syndrome is characterized by fluctuating levels of soluble proinflammatory cytokines (12) along with a decreased presence of cell-associated tumor necrosis factor receptors (TNFRs) (5). This deficit in monocyte cell surface TNF activity is associated with a poor outcome (5).

Two distinct TNFRs have been characterized, differing both in their molecular weight (55 kDa for TNFR-I, 75 kDa for TNFR-II) and in their relative expression in human cell lines. Both receptors belong to a family of cell surface proteins, including the receptors for nerve growth factor, Fas antigen, BP-50, OX-40, and CD-27, each of which exhibits a characteristic repeating extracellular cystein motif (27). Monocytes, neutrophils, and activated lymphocytes express both TNFRs (35), and the density of TNFR-II being higher than TNFR-I in resting peripheral blood monocytes and in activated T cells (6). TNFR-I shares an intracytoplasmic sequence with Fas (CD-95), a transmembrane receptor that transduces signals leading to programmed cell death (apoptosis) (26). Recent data suggest that only cells cotransfected with both TNFR-I and TNFR-II exhibit TNF-dependent apoptosis and that increased expression of TNFR-II promotes this process (30). Hence, the adequacy as well as the pattern of TNF expression may be critical for the endogenous regulation of inflammatory cell turnover.

A loss of monocyte TNFRs (moTNFRs) occurs during both experimental (10) and clinical endotoxemia (31). This postinjury deficit of membrane-associated receptors may serve to attenuate the acute effects of inflammatory cytokine products. Nevertheless, a postinjury maintenance or restoration of these membrane TNFRs actually is associated with eventual survival (5).

After severe injury, concurrent increased levels of counter-regulatory hormones, including both cortisol (2) and catecholamines (32), modulate various components of the cytokine cascade during sepsis. We have demonstrated in vitro and in vivo (32) that catecholamines inhibit TNF production during concomitant lipopolysaccharide (LPS) stimulation through β-adrenoreceptor activation. Nevertheless, it is unknown whether catecholamines and/or corticosteroids may influence the expression of moTNFR after LPS exposure. Furthermore, the specific role of β-receptor subtypes on catecholamine-elicited TNF inhibition remains unclear.

The purpose of this study was to determine whether catecholamines and/or corticosteroids may influence the appearance of moTNFR in LPS-stimulated whole blood and, if so, to determine which type of moTNFR is involved and to clarify the mechanisms of this effect. We also further dissected the role of β₁- and β₂-adrenoreceptors on catecholamine-elicited TNF down-regulation.

MATERIALS AND METHODS

Whole blood assays. Blood from healthy adults was collected aseptically using a sterile collecting system consisting of a butterfly (Butterfly, Abbott Laboratories, North Chicago, IL) connected to a needle (Becton-Dickinson, Rutherford, NJ) in a 10-ml tube containing EDTA-K₃ (vacutainer, Becton-
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Dickinson, Franklin Lakes, NJ) or in sterile heparin-prefilled (heparin sodium, 10 U/ml blood final concentration, Elkins-Sinn, Cherry Hill, NJ) 50-ml conical tubes (Sarstedt) for TNF and cytokine assessment, respectively. Whole blood for moTNFR assays was collected in EDTA tubes to avoid heparin-mediated release of TNFR (17). We studied the hormonal influences on cytokine production and moTNFR in LPS-stimulated whole blood because this in vitro model produces similar cytokine release kinetics to that observed in isolated peripheral blood mononuclear cell (PBMC) systems (8). Previous experiments demonstrated that the fluorescence signal of moTNFR remained stable for up to 12 h of incubation in the absence of LPS and that the temporal pattern of moTNFR downregulation after LPS incubation was similar to that observed in vivo (31).

To assess the influence of counter-regulatory hormones on whole blood moTNFR and TNF production, blood was diluted 1:1 in sterile RPMI-1640 supplemented with l-glutamine (Bio Whittaker, Walkersville, MA) and incubated in 5-ml sterile polypolyethylene tubes (Falcon, VWR, West Chester, PA) for up to 8 (moTNFR II) or 24 h (TNF) at 37°C and 5% of CO2, in the presence or absence of LPS (100 ng/ml final concentration; Escherichia coli serotype 0127:B8, Sigma Chemical, St. Louis, MO) or 0.9% NaCl as a control (Abbott Laboratories). Previous dose response and time sequence experiments in the presence of LPS (100 ng/ml) showed maximal total moTNFR upregulation by epinephrine (Epi), norepinephrine (NE), and dexamethasone (Dex) at 10^-6 M concentration after 8 h of incubation (data not shown). Hormonal influences on total moTNFR, moTNFR-I, and moTNFR-II were assessed at baseline and 30, 60, 180, and 360 min afterward in the presence of Epi (10^-6 M, Abbott Laboratories), Dex (10^-6 M, Elkins-Sinn), or both (EpiDex, 10^-6 M). The influence of specific adrenoreceptors on total moTNFR and TNF production were evaluated in the presence of a combination of Epi and NE (EpiNE, 10^-6 M, Abbott Laboratories) alone or along with phentolamine (α1,-antagonist, 10^-5 M; Regtine, Ciba-Geigy, Basel, Switzerland), propranolol hydrochloride (β1,- antagonist, 10^-5 M; Inderal, Ayerst, Philadelphia, PA), metoprolol tartrate (β1-agonist, dose response from 10^-5 to 10^-8 M; Research Biochemicals International, Boston, MA), the effect of adenosine 3',5'-cyclic monophosphate (cAMP) agonists on total moTNFR was tested in the presence of N^5,2'-O-dibutyryl cAMP (DBcAMP, 10^-5 M; Sigma Chemical), the type IV phosphodiesterase inhibitor Ro-20 1724/000 (28) (Ro-20; dose response from 10^-5 to 10^-7 M; generously provided by J. Jerry Sepinwall, Hoffmann-La Roche, Nutley, NJ) or a combination of both. The β2-agonist ICI-118,551 and DBcAMP were solubilized weekly in 0.9% NaCl and Ro-20 and in 50% of polyethylene glycol (Sigma Chemical) (<2% in final concentration) and stored at -70°C until assayed. Aliquots were freshly thawed for each experiment.

MoTNFR measurements. Monocyte-associated TNFR (total moTNFR) were determined as previously described (31). Briefly, erythrocytes in 400 µl of blood underwent lysis with bicarbonate-buffered (pH 7.2) 0.826% ammonium chloride solution. Leukocytes were recovered by centrifugation, washed with cold phosphate-buffered saline (PBS) containing 0.1% sodium azide and stained with 1 µg/ml biotinylated TNF. After incubation on ice for 15 min, cells were washed with cold PBS containing 0.1% sodium azide and stained with 0.5 µg/ml of streptavidin R-phycocerythrin (PE) (Caltag Laboratories, South San Francisco, CA) for 15 min on ice. Leukocytes were then washed twice with PBS containing 0.1% sodium azide and resuspended for fluorescence-activated cell sorter (FACS) analysis. Nonspecific staining was assessed by incubating only with PE-conjugate streptavidin. This method gave a background identical to that obtained when leukocytes were incubated with 100-fold excess unlabeled human TNF, as previously reported (5).

Expression of moTNFR-I (CD-120b) and moTNFR-II (CD-120a) receptors was determined using specific antibodies directed against either the TNFR-I receptor (htr-20), or the TNFR-II receptor (utr-4), both kindly donated by Dr. M. Brockhaus (F. Hoffmann-La Roche, Basel, Switzerland). Htr-20 and utr-4 are noninhibitory, nonagonist antibodies that do not interfere with the binding of TNF to its receptors (3). In experiments in which htr-20 and utr-4 were used, erythrocytes in 400-µl aliquots of blood were lysed and leukocytes were incubated with either htr-20, utr-4, or mouse immunoglobulin G1 (MOPC-21, Sigma Chemical; all 50 µl of a 20 µg/ml solution) for 45 min on ice. After being washed with PBS, leukocytes were then stained with Fab(ab)_2 fragment of PE-conjugated sheep anti-mouse antibody (Sigma Chemical; 50 µl of 14 µg/ml) for 30 min on ice. Thereafter, leukocytes were washed twice with cold PBS and resuspended for FACS analysis. The flow cytometer photomultiplier gain was standardized using PE-conjugated beads (Calibrite, Becton-Dickinson Immunochemistry Systems, San Jose, CA). Mean channel fluorescence (MCF) at >570 nm was assessed for forward and side angle light scatter-gated monocytes. Data was presented as absolute MCF units that represent the difference between MCF intensities of specifically and nonspecifically stained cells.

Assays. Levels of TNF (CLB, Amsterdam, the Netherlands) immunoactivity were measured in plasma supernatants using specific enzyme-linked immunosorbent assay. The sensitivity of this immunoassay was 4 pg/ml. Cytokine levels are expressed as nanograms per 10^6 PBMCs. Leukocyte counts and differentials were determined in K2-EDTA anticoagulated blood using FACS.

Statistical analysis. Data are summarized as means ± SE. Data were analyzed by one- or two-way analysis of variance. When variables did not follow a normal distribution, nonparametric analysis such as the Friedman test and Wilcoxon’s sum rank test were employed. Variables that did not follow a normal distribution were log transformed. When variables did not follow a normal distribution, nonparametric analysis such as the Friedman test and Wilcoxon’s sum rank test were employed.

RESULTS

After 8 h of incubation, neither Dex nor EpiDex significantly influenced the expression of moTNFR in the absence of LPS (78 ± 7, 79 ± 9, and 103 ± 12 MCF in saline, Dex, and EpiDex, respectively, P = 0.2, n = 5). The levels of TNF in non-LPS-stimulated whole blood for all whole blood experiments were 0.004 ± 0.001 ng/10^6 PBMC.

Epi increases total moTNFR by selective upregulation of moTNFR-II in LPS-stimulated whole blood. To investigate the effect of catecholamines and corticosteroids over time on total moTNFR, moTNFR-I, and moTNFR-II, whole blood was incubated in the presence of LPS alone or in combination with Epi (10^-6 M), Dex (10^-6 M), EpiDex (10^-6 M), or saline as a control, and sequentially assayed for total moTNFR, htr-20 (moTNFR-I), and utr-4 (moTNFR-II) at baseline and 30, 60, 180, and 360 min afterward and analyzed by FACS. We found that total moTNFR, moTNFR-I, and moTNFR-II were significantly diminished within 30 min after LPS.
Catecholamines increase total moTNFR and inhibit TNF production through β2-adrenoreceptor activation.

We investigated the role of the different adrenoreceptors on the expression of total moTNFR and TNF production by incubating whole blood over 8 (total moTNFR) or 24 h (TNF) in the presence or in the absence of LPS alone (100 ng/ml) or in combination with EpiNE (10^{-6} M), with and without α_{1-2}, β_{1-2}, β_{1-}, and β_{2}-adrenergic antagonists. We found that EpiNE increased total moTNFR appearance (47 ± 13 and 142 ± 32 MCF in LPS and LPS + EpiNE, respectively, P < 0.05, n = 5). This effect was completely abrogated by propranolol (142 ± 32 and 44 ± 6 MCF in LPS + EpiNE without and with propranolol, respectively, P < 0.05). In addition, the specific β_{2}-antagonist, ICI-118,551, blocked the salutary effect of EpiNE on LPS-induced downregulation of total moTNFR in a dose-dependent manner (P < 0.05; Fig. 2, top). We also documented that EpiNE significantly attenuated LPS-elicited TNF production (2.4 ± 0.3 and 0.8 ± 0.2 ng/10^6 PBMC in LPS and LPS + EpiNE, respectively, P < 0.05), and this effect was substantially abrogated by propranolol (0.8 ± 0.2 and 1.9 ± 0.4 ng/10^6 PBMC in

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Fig. 1. Study over time of different combinations of counter-regulatory hormones on whole blood lipopolysaccharide (LPS)-induced total monocyte tumor necrosis factor receptors (moTNFR; top), moTNFR-I (middle), and moTNFR-II (bottom). Changes were assessed at time 0 and 30, 60, 180, and 360 min after incubation in the presence of saline (Sal; ○) or LPS alone (●) or in combination with epi/norepinephrine (Epi, 10^{-6} M; ▲), dexamethasone (Dex, 10^{-6} M; ●), or both (EpiDex, 10^{-6} M; ■). Results are expressed as differences between specific mean channel fluorescence (MCF) and nonspecific MCF (means ± SE, n = 3). * P < 0.05, Sal vs. different groups. † P < 0.05, Epi and EpiDex vs. LPS; one- and two-way analysis of variance (ANOVA) and Fisher's post hoc protected least significant difference (PLSD).

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Fig. 2. Contribution of adrenoreceptor class on upregulation of LPS-induced moTNFRs expression (top) and TNF production inhibition (bottom). Whole blood was incubated for 8 h (total moTNFR) or 24 h (TNF) in presence of Sal (open bars), LPS alone (100 ng/ml; filled bars), or LPS along with either Epi + norepinephrine alone (EpiNE, 10^{-6} M; thick-striped bars), or in combination with α_{1-2} (thin-striped bars), β_{1-2}, β_{1-}, and β_{2}- (all gray bars) antagonists. Results are expressed as MCF units (top) and ng/10^6 peripheral blood mononuclear cell systems (PBMC; bottom) and represent means ± SE, n = 5. * P < 0.05 vs. EpiNE and † vs. LPS; one-way ANOVA and Fisher's post hoc PLSD.
LPS + EpiNE without and with propranolol, respectively, \( P < 0.05 \). Blockade with the \( \beta_2 \)-antagonist ICI-118,551 revealed similar results \( 0.8 \pm 0.2 \) vs. \( 1.8 \pm 0.4 \) ng/10^6 PBMC in LPS + EpiNE without and with \( \beta_2 \)-antagonist \( (10^{-5} \text{ M}) \), respectively, \( P < 0.05 \). The \( \beta_2 \)-antagonist metoprolol did not alter the Epi effect on TNF production \( (0.8 \pm 0.2 \) vs. \( 1.0 \pm 0.3 \) ng/10^6 PBMC in LPS + EpiNE without and with metoprolol, respectively, \( P = 0.6 \); Fig. 2 bottom). CAMP agonists partially mimic the effect of Epi on total moTNFR expression. To further investigate the intracellular pathway specific to these adrenergic influences on total moTNFR, whole blood was coincubated over 8 h in the presence of LPS alone or in combination with DBcAMP, the selective type IV phosphodiesterase inhibitor Ro-20, or a combination of both. DBcAMP only partially mimicked the above noted \( \beta \) and \( \beta_2 \) effect, because this compound significantly enhanced the LPS-elicted total moTNFR \( (32 \pm 5 \) vs. \( 51 \pm 6 \) MCF in LPS and LPS + DBcAMP, respectively, \( P < 0.05 \), \( n = 8 \)). Ro-20 alone did not influence total moTNFR when coincubated with LPS, nor did this agent significantly enhance the effect of DBcAMP \( (51 \pm 6 \) vs. \( 58 \pm 5 \) MCF in LPS + DBcAMP with and without Ro-20 \( (10^{-6} \text{ M}) \), respectively (Fig. 3).

**DISCUSSION**

This study demonstrates that \( \beta_2 \)-adrenoceptor activation not only causes inhibition of TNF production in LPS-stimulated whole blood but also fosters the restoration of moTNFRs under such circumstances. Epi enhanced moTNFR by a selective increase in the type II TNFR. This catecholamine-elicted effect was essentially prevented by a \( \beta_2 \)-antagonist. Furthermore, we observed that this influence on moTNFR upregulation was at least partially independent of TNF ligand as Dex-suppressed TNF production (results not shown) without significantly influencing either the initial or subsequent moTNFR responses to LPS upregulation.

ATNF-\( \alpha \)-converting enzyme has recently been cloned (20). This membrane-bound disintegrin metalloproteinase (MMP) processes the TNF membrane-bound precursor of 26 kDa into the soluble and mature form of 16 kDa. Furthermore, it has been demonstrated that the transmembrane receptors TNFR-II (p75) (36) and Fas ligand (14) may shed by a metalloproteinase-dependent mechanism. Furthermore, experiments in vitro have demonstrated that Epi downregulates soluble TNF through posttranscriptional mechanisms (23). Epi might increase moTNFR appearance and decrease TNF (26 kDa) shedding by modulating MMP activity. It has been demonstrated that CAMP agonists decrease MMP activity by inhibiting their synthesis (9) or by enhancing the tissue metalloproteinase inhibitor, thioinosinic acid I (25).

The effect of TNF inhibition through \( \beta_2 \)-adrenoceptor activation is in contrast to a previous report suggesting an increased TNF response after catecholamine incubation (24). In that study mouse macrophages were coincubated in the presence of UK-14304, a specific \( \alpha_2 \)-adrenergic agonist. It is possible that macrophages display a different adrenoreceptor phenotype compared with human monocytes. Along this line, a loss of \( \beta \)-receptor function during the maturation of human monocytes to macrophages has been demonstrated (1). Hence, a selective \( \alpha \)-stimulation eliciting a calcium-dependent protein kinase activation may enhance transcriptional factors induced by proinflammatory cytokines. The present data are also in contrast to previous studies of TNF inhibition through \( \beta_1 \)-activation (33). Because the main difference between both studies is the agonist employed, it is possible that, by incubating NE alone (a predominant \( \alpha_2 \) and \( \beta_1 \)-agonist) and excluding Epi, the influence of the \( \beta_1 \)-receptor might be overlooked. Both \( \beta \)-receptors stimulate adenyly cyclase and increase CAMP levels (16). Nevertheless, because \( \beta_1 \) is more resistant than \( \beta_2 \) to both short- and long-term desensitization (19), an increased density of \( \beta_2 \)-receptors in human monocytes (34) may account for the effect documented in the present study.

Epi may also enhance moTNFR synthesis, as has been demonstrated for CAMP agonists that increase TNF-\( \alpha \)-II mRNA in leukemic cells (18). In the present study we found that DBcAMP only partially reproduced the EpiNE effect. One possibility for this discrepancy is that catecholamines, by specifically binding to the receptor-G protein complex, may sustain more biologically active levels of CAMP (13) or that the adrenergic agonist-G \( \alpha \) complex elicits moTNFR upregulation by a CAMP-independent mechanism (7). Another possibility for this different response could be that, in this whole blood model, DBcAMP might be further metabolized to adenosine and dibutyrate. There is general agreement that the soluble compound DBcAMP is capable of permeating the cell membrane and mimics that action of endogenous CAMP (4). However, we cannot rule out that the effect on moTNFR might also be triggered by adenosine through a type 1 purinergic (P1, subclass A1) receptor activation (11), as the complex adenosine-A2 may also activate CAMP-depen-
dent protein kinase (15). Nevertheless, a similar effect on TNFR upregulation has been observed by employing 8-bromo cAMP, a compound that is not metabolized to other potentially active second messengers (22). Furthermore, the differences in activity between Epi and DBcAMP could not be explained by a decreased half-life of the soluble cAMP agonist, because the combination of DBcAMP with the phosphodiesterase inhibitor Ro-20 did not significantly increase the effect over that of DBcAMP alone.

In summary, this study shows that \( \beta \)2-activation increases the recovery of monocyte TNF binding capacity after LPS stimulation. This occurs via a type-II TNF receptor enhancement. Although the maximum effect was observed in response to Epi concentrations above the physiological range, these findings do suggest the potential for this mechanism during inflammatory conditions. Although it remains to be determined whether severe stress conditions are sufficient to elicit this response in vivo, it is tempting to speculate that exogenous administration of catecholamines might be of therapeutic benefit for maintenance or restoration of immune cell receptor status. The biological relevance of this study is supported by the observations that, in critically ill patients, levels of proinflammatory cytokines correlate poorly with clinical outcome, whereas monocyte and granulocyte TNF receptor levels appear to acutely reflect the ultimate prognosis of patients with severe sepsis (5). This deficit in monocyte- or granulocyte-associated receptors might inhibit TNF and/or FasL-mediated apoptosis and thereby perpetuate a dysfunctional inflammatory cell population. Also, a concomitant decreased presence of cell-associated TNF might interfere with cell-to-cell paracrine signaling and the necessary immunomodulatory effects of such activity.

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