Down-regulation of surface receptors for TNF and IL-1 on circulating monocytes and granulocytes during human endotoxemia: effect of neutralization of endotoxin-induced TNF activity by infusion of a recombinant dimeric TNF receptor

van der Poll, T.; Coyle, S.M.; Kumar, A.; Barbosa, K.; Agosti, J.M.; Lowry, S.F.

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Down-Regulation of Surface Receptors for TNF and IL-1 on Circulating Monocytes and Granulocytes During Human Endotoxemia

Effect of Neutralization of Endotoxin-Induced TNF Activity by Infusion of a Recombinant Dimeric TNF Receptor

Tom van der Poll,*† Susette M. Coyle,* Ashwini Kumar,* Karen Barbosa,* Jan M. Agosti,* and Stephen F. Lowry‡*

Leukocytes rapidly lose their surface receptors for TNF and IL-1 upon exposure to various stimuli in vitro. We sought to determine by FACS analysis changes in the expression of TNF receptors (TNFR) and type II IL-1R on circulating monocytes and granulocytes during endotoxemia in vivo, and the role of endogenous TNF in these changes. Twelve healthy subjects received an i.v. injection with LPS (2 ng/kg), directly preceded by a 30-min infusion of either a recombinant human dimeric TNFR type II-IgG fusion protein (TNFR:Fc; 6 mg/m²; n = 6) or vehicle (n = 6). LPS administration was associated with decreases in the expression of types I and II TNFR and type II IL-1R on both monocytes and granulocytes. Treatment with TNFR:Fc completely neutralized LPS-induced TNF activity (p < 0.0001 vs LPS only), modestly blunted the decrease in monocyte TNFR (p < 0.05), but did not influence reduced expression of granulocyte TNFR or monocyte/granulocyte type II IL-1R. In separate experiments, rTNF added to whole blood reduced cellular type I and type II TNFR expression by an effect on the type I TNFR; TNF did not (monocytes) decrease or only marginally (granulocytes) decrease type II IL-1R expression. LPS induces down-modulation of monocyte and granulocyte receptors for TNF and IL-1 in humans in vivo. TNF is involved in reduced monocyte TNFR expression during endotoxemia. The Journal of Immunology, 1997, 158: 1490–1497.

Sepsis and endotoxemia are associated with systemic activation of the cytokine network (1). Excessive production of the proinflammatory cytokines TNF and IL-1 importantly contributes to tissue injury and organ dysfunction in animal models in which endotoxin or live bacteria are administered i.v. (2–5). TNF and IL-1 exert their biologic effects by binding to specific cellular receptors, of which there are two distinct receptor types for each cytokine (6–8). The type I (p55) TNFR mediates most known biologic effects of TNF, including cytotoxicity (9, 10), expression of adhesion molecules on endothelial cells and leukocytes (11), and activation of nuclear factor-κB (11). Moreover, signaling via the type I TNFR is responsible for tissue injury in animals in vivo caused by either rTNF (12) or endogenous TNF produced during endotoxemia (13, 14). Stimulation of the type II (p75) TNFR results in a proliferative response of mouse thymocytes and cytotoxic T cells (9), fibroblasts, and NK cells (15). In addition, the type II TNFR may function to facilitate triggering of the type I TNFR, especially at low TNF concentrations (6, 16). The type I IL-1R signals all known cellular responses to IL-1. The type II IL-1R does not induce signal transduction, and may regulate IL-1 activity as a cell-bound decoy receptor (7, 8, 17, 18).

Regulation of the expression of transmembrane TNFR and IL-1R may represent a mechanism by which the host can influence cellular effects of these potent mediators. In vitro, leukocytes rapidly lose their ability to bind TNF and IL-1 upon exposure to various stimuli (19–31). The reduction of TNF-binding capacity of leukocytes has been linked to both shedding and internalization of TNFR (19–30), while decreased IL-1-binding capacity has been found to be associated with the release of the extracellular domain of the type II IL-1R (31, 32). Intravenous administration of endotoxin (LPS) to healthy subjects induces shedding of the extracellular ligand-binding parts of TNFR, resulting in increased plasma concentrations of soluble TNFR types I and II (30, 33, 34). We recently demonstrated that this in vivo effect of LPS coincided with a transient decrease in TNF binding by circulating monocytes and granulocytes (30). Distinct analysis of expression of type I and type II TNFR was not performed in that study.

It can be argued that TNF itself is involved in the down-modulation of surface TNFR during endotoxemia. Indeed, TNF can down-regulate its receptors on several cell types in vitro (21–23, 26–29), and i.v. injection of rTNF causes a marked reduction in TNF binding by circulating granulocytes in baboons (12). Little, if anything, is known about regulation of IL-1R expression during endotoxemia. Therefore, in the present study we sought to determine changes in the expression of surface receptors for TNF and...
IL-1 on circulating monocytes and granulocytes after i.v. administration of LPS to normal subjects, and the effect hereon of neutralization of LPS-induced TNF activity.

Materials and Methods

Study design and subjects

Twelve male subjects, aged 28 ± 2 (mean ± SE) yr, were admitted to the Adult Clinical Research Center of the New York Hospital-Cornell University Medical Center (New York, NY) 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. All subjects received an i.v. injection with LPS (National Reference Endotoxin, Escherichia coli 0113:K12 EC-5), generously provided by Dr. H. D. Hochstein, Bureau of Biologics, Food and Drug Administration, Bethesda, MD) at a dose of 2 ng/kg body weight, at 9:00 a.m. The subjects were randomized to also receive a 30-min i.v. infusion (starting at 8:30 a.m.) with either a recombinant dimeric TNFR (TNFR:Fc; Immunex Corp., Seattle, WA) at a dose of 6 mg/m² (n = 6), or vehicle (n = 6). TNFR:Fc was manufactured by fusing two identical extracellular portions of the type II TNFR with the Fc domain of IgG1 (35). The resulting dimeric TNFR binds TNF with a 50-fold greater affinity (Kd = 10⁻¹⁰ M⁻¹) than monomeric receptor (35). The serum t½ of TNFR:Fc in healthy humans is ~79 h; its clearance is ~0.5 ml/min/m² (Investigational Drug Brochure, Immunex Corp.). TNFR:Fc was reconstituted with 1 ml of sterile water for injection from a lyophilized powder containing 10 mg of TNFR:Fc; 1.2 mg of Tris (trimethamine), 10 mg of sucrose, and 40 mg of mannitol. The final dose of TNFR:Fc (or vehicle) was diluted in 100 ml of isotonic saline before infusion. Two hours before the administration of LPS, a radial arterial catheter was placed in all subjects to continuously monitor heart rate and blood pressure (Datascope model 2000A; Datascope Corp., Paramus, NJ), and for blood sampling. A rectal probe was inserted to allow continuous measurement of core temperature. Arterial blood was obtained at 8:30 a.m. (i.e., directly before the start of the infusion with TNFR:Fc or vehicle, t = −0.5 h), directly before the injection of LPS (t = 0 h), and 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, and 24 h thereafter. All blood samples (except samples for flow cytometry) were centrifuged at 4°C for 20 min at 1600 × g and stored at −70°C until assayed.

Whole blood stimulation

For each whole blood stimulation, blood was collected aseptically from five different healthy donors in sterile tubes prefilled with K₂-EDTA (Becton Dickinson, Rutherford, NJ), and aliquots of 3 ml of whole blood were added to sterile polystyrene tubes (Becton Dickinson). These aliquots were then incubated at 37°C for various time periods and at various concentrations (see Results) with one of the following reagents: sterile isotonic saline (diluent), human rTNF (kindly donated by Dr. T. Kohno, Amgen, Boulder, CO), or TNF mutants (p55-TNF and p75-TNF), with exclusive affinity for either the type I TNFR or the type II TNFR, respectively (both kindly donated by Dr. W. Lesslauer, F. Hoffmann-La Roche, Basel, Switzerland). The generation of the p55- and p75-TNF mutants has been described previously (10). After the incubation, blood samples were processed for flow cytometry, as described below.

Flow cytometry

Leukocyte counts were determined by flow-cytometric light scatter analysis. During in vivo studies, saturation binding of TNF by monocytes and granulocytes was determined by FACScan analysis, as described previously (12, 30). Expression of type I and type II TNFR was determined using specific Abs directed against either the type I TNFR (htr-20), or the type II TNFR (utr-4), both kindly donated by Dr. M. Brockhaus (F. Hoffmann-La Roche) (33, 36, 37). htr-20 and utr-4 are noninhibitory Abs that do not interfere with the binding of TNF to its receptors. Hence, addition of high amounts of TNF to cells is unlikely to influence the binding of htr-20 and utr-4 to the type I and type II TNFR, respectively (33, 36, 37). Expression of the type II IL-1R was determined using a specific Ab directed against this receptor species (M2S), kindly donated by Dr. J. E. Sims (Immunex Corp.) (31). In experiments in which htr-20, utr-4, or M2S was used, erythrocytes were washed with PBS-azide, which are usually included in the experiments, and leukocytes were incubated with htr-20, utr-4, M2S, or mouse IgG1 (MOPC-21; Sigma Chemical Co., St. Louis, MO) (all 50 μl of a 20 μg/ml solution) for 45 min on ice. After a washing with cPBS-azide, leukocytes were then stained with a F(ab)² fragment of phycoerythrin-conjugated sheep anti-mouse IgG (Sigma Chemical Co.) for 30 min on ice. Thereafter, leukocytes were washed with cPBS-azide and resuspended for flow cytometric analysis. The flow-cytometer photomultiplier gain was standardized using phycoerythrin-conjugated beads (Calibrite; Becton Dickinson Immunocytometry Systems, San Jose, CA). Forward and side angle scatter-gated monocytes and granulocytes was assessed. Data are presented as the difference (linear units) between MCF intensities of specifically and nonspecifically stained cells.

Assays

Plasma TNF activity was determined using the WEHI 164 clone 13 fibroblast cytotoxicity assay (38), as described previously (39). The limit of detection of the assay was 20 pg/ml. Plasma TNF immunoreactivity was determined by ELISA (CLB Biotechnology Department, Amsterdam, The Netherlands), with a limit of detection of 5 pg/ml (40). Plasma concentrations of soluble TNFR (reagents kindly provided by Dr. W. A. Buurman, University of Limburg, Maastricht, The Netherlands) were measured by ELISA (24, 25). Soluble IL-1R type II was measured by ELISA essentially as described (31). Anti-IL-1R type II mAb M2 (2.5 μg/ml) was used as capturing Ab, and rabbit polyclonal anti-IL-1R type II followed by donkey anti-rabbit IgG-horsedard peroxidase were used for detection. Purified soluble rIL-1R type II was used as standard (31). The reagents for the soluble IL-1R type II ELISA were kindly donated by Dr. J. E. Sims (Immunex Corp.).

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 for matched samples. p < 0.05 was considered to represent a statistically significant difference.

Results

TNF release

LPS induced a transient increase in plasma TNF activity, peaking after 1.5 h (0.219 ± 0.042 ng/ml; p < 0.0001 vs baseline) (Fig. 1). TNF activity remained undetectable in subjects infused with TNFR:Fc (p < 0.0001 vs LPS only). Furthermore, addition of 10 ng/ml of rTNF to plasma samples obtained from TNFR:Fc-treated subjects at 1.5 and 24 h after injection of LPS did not result in detectable TNF activity, indicating that the amount of TNFR:Fc administered offered an excess of TNF-neutralizing capacity. Plasma TNF immunoreactivity also showed a monophasic rise in subjects injected with LPS only, peak levels being reached after 1.5 h (0.602 ± 0.121 ng/ml; p < 0.0001 vs baseline) (Fig. 1). In this treatment group, TNF immunoreactivity was undetectable again at 3 to 4 h postinjection. By contrast, high levels of TNF immunoreactivity were present in plasma from subjects infused with TNFR:Fc for up to 24 h after LPS injection (p = 0.0001 vs LPS only). TNF reached a plateau between 3 and 8 h (4 h: 10.489 ± 3.015 ng/ml).

Clinical responses

LPS administration was associated with a rise in body temperature in both study groups (p < 0.0001 vs baseline). However, in subjects treated with TNFR:Fc, the febrile response was blunted significantly when compared with subjects injected with LPS only (p < 0.0001 for the difference between groups; Fig. 2). Peak temperatures were 37.7 ± 0.1°C and 38.2 ± 0.1°C, respectively. In addition, the increase in pulse rate was attenuated by TNFR:Fc when compared with subjects treated with LPS only (p = 0.05 for the difference between groups; Fig. 2). Mean arterial blood pressure did not change in either group (data not shown).

TNF receptors

Changes in cellular TNF expression are given in Figure 3. Injection of LPS only induced a transient decrease in TNF binding by circulating monocytes, reaching a nadir at 2 h in all volunteers (p < 0.0001 vs baseline). Decreased monocyte TNF binding was associated with a reduced expression of both type I and type II TNFR at the surface of monocytes, also reaching nadirs at 2 h.
TNF binding by circulating granulocytes, reaching a nadir after 4 h (both \( p < 0.0001 \) vs baseline). In subjects infused with TNFR:Fc, LPS-induced decreases in monocyte TNF binding and monocyte type I and type II TNFR expression were all blunted significantly (both \( p < 0.05 \) vs LPS only).

Administration of LPS only elicited a more sustained decline in TNF binding by circulating granulocytes, reaching a nadir after 4 h (both \( p < 0.0001 \) vs baseline), and associated with a decreased expression of type I (both \( p = 0.0001 \)) and type II TNFR (both \( p = 0.09 \)) on granulocytes. Although treatment with TNFR:Fc partially attenuated these LPS-induced changes on granulocytes, the differences with subjects injected with LPS only did not reach statistical significance.

LPS-induced rises in the plasma concentrations of type I and type II soluble TNFR, peaking after 2 h (3.63 ± 0.36 and 7.26 ± 0.94 ng/ml, respectively; both \( p < 0.0001 \) vs baseline). TNFR:Fc attenuated the LPS-induced rise in the type I soluble TNFR (both \( p < 0.0001 \) vs LPS only), peak levels being measured slightly later than in subjects injected with LPS only (3 h: 2.64 ± 0.37 ng/ml; Fig. 4). The effect of TNFR:Fc on type II soluble TNFR release could not be determined due to interference of TNFR:Fc (dimeric type II TNFR) with the assay.

Type II IL-1R

LPS elicited decreases in the expression of type II IL-1R at the surface of both circulating monocytes (both \( p = 0.001 \) vs baseline) and granulocytes (both \( p < 0.0001 \)), nadirs being measured after 2 h (Fig. 5). TNFR:Fc influenced LPS-induced changes in type II IL-1R expression neither on monocytes nor on granulocytes.

Injection of LPS did not induce changes in the plasma concentrations of the type II soluble IL-1R in either treatment group (Fig. 4).

**FIGURE 1.** Mean (± SE) plasma concentrations of TNF activity and TNF immunoreactivity after i.v. injection of LPS (lot EC-5, 2 ng/kg) at \( t = 0 \) h in subjects receiving either a 30-min infusion of TNFR:Fc (6 mg/mL; open circles; \( n = 6 \)) starting at \( t = -0.5 \) h, or vehicle (closed circles; \( n = 6 \)). \( p \) value indicates the difference between treatment groups.

**FIGURE 2.** Mean (± SE) rectal temperature and pulse rate after i.v. injection of LPS (lot EC-5, 2 ng/kg) at \( t = 0 \) h in subjects receiving either a 30-min infusion of TNFR:Fc (6 mg/mL; open circles; \( n = 6 \)) starting at \( t = -0.5 \) h, or vehicle (closed circles; \( n = 6 \)). \( p \) value indicates the difference between treatment groups.

Whole blood stimulation

We next sought to determine whether TNF can influence cellular TNFR and IL-1R expression. Incubation of human whole blood with human rTNF (10 ng/ml) resulted in a rapid decrease in type I and type II surface TNFR on monocytes and granulocytes, maximal effects being measured after 60 min (Fig. 6). The down-regulation of transmembrane TNFR is associated with a modest rise of doublet physiologic relevance) in soluble TNFR in supernatant plasma (Table I). The effect of TNF on monocyte and granulocyte TNFR was dose dependent (data not shown). By contrast, TNF did not influence the expression of type II IL-1R on monocytes, and only modestly down-modulated the expression of this receptor species on granulocytes (Fig. 6). Addition of higher doses of TNF (up to 100 ng/ml) for longer time periods (up to 4 h) did not influence type II IL-1R expression further (data not shown). In addition, TNF did not affect levels of soluble IL-1R type II in supernatant plasma (Table I).

To determine which TNFR signals the effect of wild-type TNF on the expression of TNFR on monocytes and granulocytes, we next incubated whole blood with TNF mutants with specific affinity for either the type I TNFR (p55-TNF, 10 ng/ml) or the type II TNFR (p75-TNF, 10 ng/ml). p55-TNF was added at a dose of 10-fold higher than p55-TNF, because of its 10-fold lower affinity for the p75 receptor than wild-type TNF (10). p55-TNF decreased the expression of both type I and type II TNFR on monocytes and granulocytes, while p75-TNF had no effect (Fig. 7). Neither p55-TNF nor p75-TNF influenced the expression of IL-1R type II on these cell types (Fig. 7).

**Discussion**

Knowledge of factors that regulate TNFR and IL-1R expression in vivo is highly limited. Recently, we reported that administration of
LPS to normal subjects resulted in a transient reduction in TNF binding by circulating monocytes and granulocytes (30). The present findings confirm these data, and further show that this reduced TNF binding is paralleled by a down-modulation of both the type I and type II TNFR. In addition, we demonstrate for the first time that i.v. injection of LPS into healthy humans is associated with a down-regulation of type II IL-1R on circulating monocytes and granulocytes. Neutralization of LPS-induced TNF activity partially blunted the decrease in surface expression of monocyte TNFR, while the down-regulation of granulocyte TNFR and monocyte/granulocyte type II IL-1R was not influenced significantly. These data suggest that endogenously produced TNF is involved in reduced TNFR expression on monocytes during endotoxemia.

In this study, TNF activity generated in response to i.v. LPS was neutralized effectively by TNFR:Fc, as reflected by undetectable TNF activity in humans infused with this construct, and the fact that plasma obtained 1.5 or 24 h after LPS administration demonstrated a large capacity to buffer exogenously added rTNF. Apparently, TNFR:Fc bound all free circulating TNF, as indicated by high concentrations of immunoreactive TNF in TNFR:Fc-treated subjects, which may be explained by a decreased clearance of TNF due to the carrier function and prolonged t½ of TNFR:Fc (35, 41). Recently, TNFR:Fc, given at higher doses (10 and 60 mg/m²) than in the present study (6 mg/m²), was also reported to neutralize TNF activity after injection of LPS at 4 ng/kg (vs 2 ng/kg in our study) into normal humans (41). Similar to our study, TNF activity remained neutralized during a 24-h period after LPS injection, thereby making the occurrence of delayed and prolonged release of TNF activity from TNF-TNFR:Fc complexes, as has been reported in a mouse model of lethal Gram-negative sepsis (42), unlikely. Remarkably, in the previous human study it was noted that as the dose of TNFR:Fc increased, the anti-inflammatory effect decreased. Indeed, cytokine and stress hormone release were more inhibited by the lower dose than the higher dose of TNFR:Fc (41). Since the lower TNFR:Fc dose used in the earlier volunteer study still provided a large excess of TNF-neutralizing capacity (41), we chose to administer TNFR:Fc at 6 mg/m².

Despite the fact that TNFR:Fc neutralized endogenous TNF activity, this treatment only partly reduced the febrile response and the increase in pulse rate after administration of LPS. In the earlier
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FIGURE 4. Mean (± SE) plasma concentrations of soluble TNFR type I, soluble TNFR type II, and soluble IL-1R type II after i.v. injection of LPS (lot EC-5, 2 ng/kg) at \( t = 0 \) h in subjects receiving either TNFR:Fc (6 mg/m\(^2\); open circles; \( n = 6 \)) starting at \( t = -0.5 \) h, or vehicle (closed circles; \( n = 6 \)). Results are given as the difference between specific MCF and nonspecific MCF (mean ± SE). NS = not significant for the difference between treatment groups. Plasma levels of soluble TNFR type II could not be determined in subjects infused with TNFR:Fc due to interference of this construct with the assay.

human study in which TNFR:Fc was given at higher doses, TNFR:Fc delayed but did not diminish the febrile response, and did not alter the elevated heart rate (41), suggesting that indeed our lower dose exerted more potent anti-inflammatory effects. Conceivably, other mediators induced by LPS that were not or only in part inhibited by TNFR:Fc can sustain these responses. In this context, it is of interest that TNFR:Fc reduced but did not prevent the release of other cytokines in the previous human study (41), as well as in our study (data not shown).

TNF has been found to induce down-modulation of both the type I and type II TNFR on several cell lines and cell types in vitro, including isolated monocytes and granulocytes (21–23, 26, 29). Reports on via which TNFR TNF signals this effect are conflicting. Using agonistic Abs directed against one of the two TNFR species, selective triggering of the type I TNFR was found to down-regulate both TNFR types on human adherent neutrophils, while stimulation of the type II TNFR did not influence the expression of the type I TNFR (29). By contrast, in the U937 histiocytic cell line, Abs to either types of TNFR not only down-regulated their own receptors, but also reciprocal ones (21). In the present study, we stimulated whole blood and performed FACS analysis on unseparated leukocytes to avoid potential artifacts caused by isolation procedures. In this in vitro system, we clearly demonstrated the capacity of TNF to down-regulate both TNFR species on monocytes and granulocytes. By using TNF mutant proteins with specific affinity for only one of the receptors, we further showed that this TNF effect is signaled exclusively through the type I TNFR. In addition to these in vitro data, TNF infusion into baboons also reduced TNFR expression on granulocytes in vivo (12). However, neutralization of TNF activity by TNFR:Fc only partially blunted the LPS-induced decrease in monocyte TNFR expression, while granulocyte TNFR expression was not influenced significantly. Many different agonists can reduce the expression of cell surface TNFR (20, 27). Thus, although TNF may cause down-regulation of surface TNFR, it is not necessary for this response during endotoxemia, and other factors besides TNF most likely play a role, including LPS itself (19, 24, 30).

Both receptor shedding and internalization have been implicated as mechanisms of reduced TNFR expression (19–29). Administration of LPS to humans induces an increase in the plasma concentrations of soluble TNFR, suggesting that at least part of the reduced cellular TNFR expression is related to shedding (30, 33, 34, 41). In this study, we demonstrate that neutralization of TNF resulted in reduced release of soluble TNFR type I, which is in line
with primate studies of low grade endotoxemia and lethal bacteremia using mAbs as anti-TNF strategy (43–45). TNFR:Fc administered at higher doses only delayed the appearance of soluble TNFR type I, which may have been related to the paradoxical reduction of anti-inflammatory effects with increasing doses of TNFR:Fc (41). A role of TNF in the release of soluble TNFR is further supported by the fact that TNF can induce such a response in humans in vivo (43, 46).

Although IL-1 signals its biologic effects exclusively via the type I IL-1R, the majority of cell types express predominantly type II IL-1R at their surface. Indeed, type II IL-1R can be detected in only minute amounts on monocytes and granulocytes (7, 8, 17, 18, 30, 31, 47), and their expression seems not to be influenced by inflammatory agents (31). In accordance, using a panel of four different anti-IL-1R type I Abs, we were only able to detect monocyte and granulocyte expression of this receptor species by FACS analysis in a subset of healthy humans. In these subjects, the expression of the type I IL-1R was not altered consistently by LPS or TNF (data not shown). The type II IL-1R does not induce signal transduction, and can regulate IL-1 activity either as a cell-bound decoy receptor or as a soluble receptor generated by shedding of its ligand-binding extracellular domain (7, 8). Recently, LPS and TNF (10 ng/ml) were reported to reduce the expression of the type II IL-1R on neutrophils in vitro (31). In the present study, we showed

Table I. Mean (± SE) concentrations of soluble TNF receptors and soluble type II IL-1 receptor in supernatant plasma after incubation of whole blood with recombinant TNF

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Soluble TNF-R-I (ng/ml)</th>
<th>Soluble TNF-R-II (ng/ml)</th>
<th>Soluble IL-1R-II (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.67 ± 0.08</td>
<td>1.83 ± 0.15</td>
<td>6.06 ± 1.30</td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.72 ± 0.08</td>
<td>1.80 ± 0.18</td>
<td>5.67 ± 1.24</td>
</tr>
<tr>
<td>60</td>
<td>0.76 ± 0.08</td>
<td>1.71 ± 0.18</td>
<td>5.64 ± 1.13</td>
</tr>
<tr>
<td>120</td>
<td>0.83 ± 0.09</td>
<td>1.87 ± 0.16</td>
<td>5.74 ± 0.94</td>
</tr>
<tr>
<td>TNF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>0.78 ± 0.10*</td>
<td>2.06 ± 0.21*</td>
<td>6.07 ± 1.11</td>
</tr>
<tr>
<td>60</td>
<td>0.85 ± 0.10*</td>
<td>2.10 ± 0.20*</td>
<td>5.51 ± 1.00</td>
</tr>
<tr>
<td>120</td>
<td>0.91 ± 0.09*</td>
<td>2.16 ± 0.21*</td>
<td>5.81 ± 1.10</td>
</tr>
</tbody>
</table>

*Whole blood was incubated with saline or recombinant TNF (10 ng/ml) for indicated time periods at 37°C, after which plasma was obtained by centrifugation. *p < 0.05 vs saline.
that LPS reduces type II IL-1R expression on both monocytes and granulocytes in vivo, and that neutralization of TNF did not influence this response. Furthermore, incubation of whole blood with TNF at concentrations up to 100 ng/ml only modestly down-modulated the expression of this receptor species on granulocytes, while not affecting monocyte receptor expression. Thus, TNF does not seem to play an important role in the regulation of type II IL-1R expression. It should be noted that TNF can influence IL-1 activity by mediating the release of IL-1β and IL-1 receptor antagonist (48, 49). It remains to be established whether the down-modulation of surface type II IL-1R during endotoxemia is the result of shedding or internalization of receptors. The unaltered plasma levels of soluble IL-1R type II may have been related to the relatively mild challenge and/or the possibility that putative shedding of IL-1R by circulating monocytes and granulocytes does not induce significant changes in the large pool of soluble IL-1R in plasma that may be derived from cells not present in the circulation.

The role of endogenous TNF in LPS-induced changes in TNFR and IL-1R expression was not studied in whole blood in vitro, since previous reports have indicated that in vitro, the respective kinetics of loss of surface receptors (occurring very fast) and TNF production (occurring relatively late) in response to LPS make a role for endogenous TNF in these LPS effects in in vitro systems unlikely (19, 30, 31). In accordance, TNF was found not to be responsible for the loss of TNFR from the surface of RAW264.7 cells after incubation with LPS (19).

Experimental endotoxemia in humans is associated with a down-modulation of surface receptors for TNF and IL-1 on circulating monocytes and granulocytes. Down-regulation of TNFR may represent a mechanism to protect the host against excessive activity of this proinflammatory cytokine. Endogenously produced TNF plays a modest role in the down-modulation of monocyte TNFR during endotoxemia, but is not involved significantly in the reduced expression of granulocyte TNFR or the decreased expression of type II IL-1R on monocytes and granulocytes.

References


