TNF-α increases sensitivity to LPS in chronically catheterized rats

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Goto, Masakatsu, Lucy V. Deriy, Yong J. Chen, David W. A. Beno, Michael R. Uhing, Vanida A. Jiyamapa-Serna, and Robert E. Kimura. TNF-α increases sensitivity to LPS in chronically catheterized rats. Am J Physiol Heart Circ Physiol 280: H2857–H2862, 2001.—Patients with severe trauma injury are transiently exposed to increased serum concentrations of tumor necrosis factor-α (TNF-α). These patients are susceptible to the development of multisystem organ failure (MSOF) triggered by subsequent exposure to bacterial toxins either via infection or increased intestinal permeability. We simulated the cytokine response of trauma by infusing 0.8 or 8.0 μg/kg of TNF-α (priming dose) into chronically catheterized rats. After 48 h, rats were challenged with endotoxin [lipopolysaccharide (LPS); 10 or 1,000 μg/kg]. Animals primed with either dose of TNF-α and then challenged with 1,000 μg/kg of LPS demonstrated significantly increased mortality, mean peak serum concentrations of interferon-γ (IFN-γ), and blood lactate concentrations (P < 0.05) compared with nonprimed means. Mean peak serum concentrations of IFN-γ and blood lactate concentrations were increased after challenge with 10 μg/kg of LPS only in animals primed with 8.0 μg/kg of TNF-α. Priming with TNF-α did not increase mortality after challenge with 10 μg/kg of LPS. These data suggest that both TNF-α release and the subsequent exposure to bacterial toxins mediate the pathophysiological progression from trauma to subsequent MSOF.

MULTISYSTEM ORGAN FAILURE (MSOF) occurs in 11–28% of trauma patients (7, 14, 19, 20, 25). Although the etiology of MSOF is not known, the development of MSOF typically occurs 48 h after trauma and is associated with bacterial infection (28). MSOF is initiated by an uncontrolled cascade of immune inflammatory mediators induced from either the initial insult or from a subsequent second insult (the “two-hit” inflammatory model). We hypothesized that the release of the proinflammatory cytokine tumor necrosis factor-α (TNF-α), which accompanies noninfectious events such as trauma, burn injury, hypovolemia, and postoperative stress (24), enhances or primes the immune response and therefore mediates the development of MSOF.

Although the clinical findings of trauma-induced TNF-α release and subsequent bacterial infection leading to MSOF in humans are well documented, animal studies have not corroborated these observations. Previous animal studies have shown that TNF-α pretreatment attenuates lipopolysaccharide (LPS) and sepsis-induced mortality and hemodynamic and biochemical changes (1, 2, 5, 9, 15, 23). However, the results of these animal studies may be methodologically limited. First, the animals in these previous studies were examined under conditions of surgical and nonsurgical stress. Second, although MSOF usually begins within 48 h of posttraumatic injury, in these models TNF-α was administered 24 h before the onset of sepsis or the administration of LPS. Finally, in studies using rodent models, the doses of TNF-α and LPS utilized were very high, ranging from 10–200 μg·kg⁻¹·day⁻¹ and 10–20 mg/kg, respectively, which are likely beyond the concentrations encountered clinically.

To better understand the relationship between posttraumatic TNF-α release and the systemic inflammatory response that leads to the development of MSOF, we examined the effect of TNF-α administration on subsequent LPS-induced inflammatory response. Using a chronically catheterized rat model to avoid the adverse effects of surgically and nonsurgically induced stress, we administered pathophysiological concentrations of TNF-α 48 h before endotoxin administration to mirror the clinical course of MSOF patients. We used four separate measures to evaluate changes in the LPS-induced inflammatory response that was induced by pretreatment with TNF-α. We chose to measure the cytokines TNF-α and interferon-γ (IFN-γ) because of the known relationship of these cytokines with LPS-induced shock models; likewise, we chose to quantify blood lactate concentrations ([lactate]) because of the known correlation with severity and outcome of septic shock in patients with MSOF (29). Our final measure was animal survival because prevention of mortality is the ultimate goal of septic shock research.

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MATERIALS AND METHODS

Table 1. TNF-α priming and LPS challenge

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
<th>n</th>
<th>Priming (Day 1)</th>
<th>Challenge (Day 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>A</td>
<td>9</td>
<td>No treatment</td>
<td>2 ml/kg saline</td>
</tr>
<tr>
<td>LT+ HT</td>
<td>B</td>
<td>5</td>
<td>0.5 µg/kg TNF-α</td>
<td>8.0 µg/kg TNF-α</td>
</tr>
<tr>
<td>HT+ HT</td>
<td>C</td>
<td>10</td>
<td>8.0 µg/kg TNF-α</td>
<td>8.0 µg/kg TNF-α</td>
</tr>
<tr>
<td>LL</td>
<td>D</td>
<td>8</td>
<td>No treatment</td>
<td>10 µg/kg LPS</td>
</tr>
<tr>
<td>LT + LL</td>
<td>E</td>
<td>9</td>
<td>0.5 µg/kg TNF-α</td>
<td>10 µg/kg TNF-α</td>
</tr>
<tr>
<td>HT + LL</td>
<td>F</td>
<td>6</td>
<td>8.0 µg/kg TNF-α</td>
<td>10 µg/kg LPS</td>
</tr>
<tr>
<td>HL</td>
<td>G</td>
<td>8</td>
<td>No treatment</td>
<td>1,000 µg/kg LPS</td>
</tr>
<tr>
<td>LT + HL</td>
<td>H</td>
<td>12</td>
<td>0.5 µg/kg TNF-α</td>
<td>1,000 µg/kg LPS</td>
</tr>
<tr>
<td>HT + HL</td>
<td>I</td>
<td>13</td>
<td>8.0 µg/kg TNF-α</td>
<td>1,000 µg/kg LPS</td>
</tr>
</tbody>
</table>

n = No. of rats. Animals were divided into nine groups and given parenteral infusions of tumor necrosis factor-α (TNF-α) and/or lipopolysaccharide (LPS; Escherichia coli 0111:B4) 4–7 days postoperatively. LT, low-dose TNF-α (0.8 µg/kg); HT, high-dose TNF-α (8.0 µg/kg); LL, low-dose LPS (10 µg/kg); HL, high-dose LPS (1,000 µg/kg).

RESULTS

Clearance of the TNF-α priming dose. Parenteral infusions of TNF-α at 0.8 µg/kg (low TNF-α; group B) or 8.0 µg/kg (high TNF-α; group C) resulted in significant mean peak serum increases that were cleared in a time- and dose-dependent manner by 120 min (see Fig. 1). Mean peak serum [TNF-α] values 1 min post-infusion were 5.9 ± 2.5 and 178.0 ± 33.1 ng/ml for the low-TNF-α (group B) and high-TNF-α (group C).

Statistical analysis. Mean and SE of the mean are reported for all values. For all rats, the peak serum [TNF-α] and [IFN-γ] values occurred at 90 and 240 min, respectively, and mean peak blood [lactate] measurements occurred at 180 min. Mean data from these time points for each group were used for intergroup comparisons. Two-way repeated-measures ANOVA with Newman-Keuls post hoc correction were used for statistical comparison. The correlation of mortality with mean peak blood [lactate] and peak serum [IFN-γ] was determined by logistic regression with backward elimination using SPSS 10 for Windows (SPSS, Chicago, IL). Significance was accepted at P ≤ 0.05 for all measures.

Fig. 1. Serum tumor necrosis factor-α concentration ([TNF-α]) after TNF-α administration. Serum [TNF-α] values were measured at indicated time points after parenteral infusion of recombinant TNF-α (rTNF-α) at 8.0 or 0.8 µg/kg. Data are means ± SE.
Effect of priming on LPS-induced cytokine concentration and [lactate]. The mean peak serum [IFN-γ] (see Fig. 2) and blood [lactate] values (see Fig. 3) after infusion of 1,000 μg/kg of LPS were significantly increased in animals primed with either low-dose (group H) or high-dose (group I) TNF-α compared with unprimed rats (group G). In contrast, there was no difference between mean peak serum [TNF-α] values in primed (groups H and I) or unprimed (group G) rats after infusion of 1,000 μg/kg of LPS (see Fig. 4). There was no difference between mean peak serum [TNF-α] values in primed (groups E and F) or unprimed (group D) rats after infusion of 10 μg/kg of LPS (see Fig. 4). However, rats primed with high-dose TNF-α (group F) but not low-dose TNF-α (group E) demonstrated increased mean peak serum [IFN-γ] (see Fig. 2) and blood [lactate] (see Fig. 3) after infusion of 10 μg/kg of LPS compared with non-primed rats (group D).

Effect of TNF-α priming on TNF-α challenge. The mean peak serum [TNF-α] values 1 min after a second rTNF-α challenge with high-dose rTNF-α were 175.3 ± 37.4 and 27.9 ± 3.5 ng/ml for rats originally primed with low-dose (group B) and high-dose (group C) rTNF-α, respectively. There was a significant decrease in mean peak serum [TNF-α] values between the initial infusion (priming dose) of 8 μg/kg of rTNF-α (178 ± 33.1 ng/ml) and the subsequent infusion of 8 μg/kg of rTNF-α 48 h later (27.9 ± 3.5 ng/ml) for group C rats. In contrast, there was no difference between mean peak serum [TNF-α] after the initial infusion of high-dose rTNF-α (178.0 ± 33.1 ng/ml) and the mean peak values in those primed with low-dose rTNF-α and then challenged with high-dose rTNF-α 48 h later (group B; 175.3 ± 37.4 ng/ml).

Effect of priming on LPS-induced mortality. Mortality in rats primed with low-dose (group H) or high-dose (group I) TNF-α after challenge with 1,000 μg/kg of LPS was 25% (3 of 12 rats) and 38% (5 of 13 rats), respectively. No deaths occurred in any other experimental group. Furthermore, there was a positive correlation between increases in serum [IFN-γ] and blood [lactate] (see Fig. 5). The relationship between these measures and mortality was determined by logistic regression with backward elimination. When both lactate and IFN-γ were included, lactate was statistically significant (P = 0.001) but IFN-γ was not (P = 0.169); however, when analyzed independently, both lactate
and IFN-γ were highly significant predictors of mortality (lactate, \( P = 0.0005 \); IFN-γ, \( P = 0.005 \)) for these groups only. When all rats from all groups were analyzed together, neither lactate or IFN-γ were significant indicators of mortality (\( P = 0.224 \) and 0.139, respectively). Although death occurred in 8 of 25 rats in the rTNF-α primed and 1,000 \( \mu g/kg \) of LPS challenged rats (groups H and I), only 5 of the 8 are represented in Fig. 5 because the 3 other rats died before the 4-h blood samples were obtained for measurement of peak serum [IFN-γ].

**DISCUSSION**

These data demonstrate that TNF-α exposure 48 h before administration of sublethal doses of LPS significantly increases LPS-induced mortality, serum [IFN-γ], and blood [lactate], and, although it was not significant, there was also a trend toward higher LPS-induced TNF-α release. The morbidity and mortality observed in these animals were most pronounced in animals challenged with the higher TNF-α and LPS doses. Because the priming doses of TNF-α were rapidly cleared from rats and were undetectable by 120 min, the enhancement of the LPS-induced response 48 h later was the result of the TNF-α priming rather than a direct synergistic interaction between the administration of rTNF-α and the subsequent LPS challenge.

In contrast to other previous studies, our study is the only one demonstrating that pretreatment with TNF-α increases LPS-induced mortality. Both Sheppard and colleagues (23) and Alexander and co-workers (2) reported that 10 or 50 \( \mu g/kg \) of rTNF-α significantly decreased mortality in rats challenged with 10 mg/kg of LPS. In a separate study, Alexander and co-workers (1) showed that 200 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) of rTNF-α for 6 days decreased mortality after cecal ligation and puncture in rats. Similarly, Fraker and colleagues (9) reported that 200 \( \mu g \cdot kg^{-1} \cdot day^{-1} \) of rTNF-α for 3 or 5 days decreased mortality in rats after challenge with 10 mg/kg of LPS. Finally, using a pig model, Murphey and Traber (15) demonstrated that lower doses of rTNF-α (0.5 \( \mu g/kg \)) also prevented LPS-induced mortality and hypotension.

Several factors may account for the discrepancy between the results of our study and the results of previous animal studies examining LPS challenge after TNF-α exposure. First, we pretreated the animals 48 h before administration of LPS as compared with the 24-h pretreatment in previous animal studies. We selected the 48-h time period because the onset of MSOF usually occurs 48 h after TNF-α release (14, 28). A study by Fraker and colleagues (9) is the only other animal model system where LPS was administered 48 h after TNF-α treatment. These investigators found that TNF-α was protective at 200 \( \mu g/kg \cdot day^{-1} \) but that the protective effect was lost at a lower TNF-α infusion rate of 50 \( \mu g/kg \cdot day^{-1} \). In their study, however, TNF-α treatment was administered over the course of 3 or 5 days, which precludes comparisons to our model.

The second major difference between our study and previous studies in rodents is that the doses of LPS and TNF-α administered to the animals were much lower in our study. In previous studies, the doses of TNF-α ranged from 10–200 \( \mu g/kg \cdot day^{-1} \) compared with our study in which 0.8 or 8 \( \mu g/kg \) of TNF-α was administered as a single bolus. We chose these dosages to mimic the serum [TNF-α] values associated with patients after trauma (low dose) and to mimic the highest serum [TNF-α] achieved after LPS administration in our nonstressed rat model (high dose) (4, 13, 24). In addition to using larger TNF-α doses, most previous studies have also used much larger doses of LPS (10–20 mg/kg). We examined the effect of TNF-α pretreatment when smaller, nonlethal doses of LPS were administered. Similar to when we used two doses of TNF-α to simulate severity of trauma, we used two doses of LPS (10 and 1,000 \( \mu g/kg \)) to mimic differing severity of posttrauma infection.

Finally, the animals in previous studies were examined under conditions of surgical and nonsurgical stress. Surgical and nonsurgical stress are associated with elevated concentrations of catecholamines and glucocorticoids. Elevations in either of these hormones can significantly alter the inflammatory response (4, 24). We have previously shown in our model system that surgical and nonsurgical stress significantly attenuate the LPS-induced TNF-α responses (4). Therefore the use of a nonstressed model system provides a more clinically relevant model for endotoxemia than other rodent models.

The findings of our studies suggest that TNF-α may be one of the mediators involved in priming the immunological system by either initiating or perpetuating MSOF after noninfectious insults such as trauma. Patients with severe trauma exhibit increased serum [TNF-α] values and are predisposed to the development of MSOF and death triggered by normally unremarkable infections (6, 10, 13). The studies of Moore
and colleagues (14) found that bacterial infections triggered or worsened MSOF in 22% of patients with early MSOF (3 days after the traumatic event) and 32% of patients with late-onset MSOF. Waydhas and co-workers (28) reported that infection triggered the development of MSOF in 44% of patients. The source of these infections is often difficult to determine. However, multiple studies have implicated the intestine as a potential source of bacterial translocation and toxin release during the trauma-induced response (8, 18, 27).

We speculate that the development of MSOF may be the result of trauma-induced increases in TNF-α which prime the host to become more sensitive to bacterial toxins including LPS. This is emphasized by our data revealing that 1,000 μg/kg of LPS induced 25–38% mortality in TNF-α primed rats whereas other studies require >10 mg/kg to induce similar mortality (9, 22). Therefore, in trauma patients, even a minor insult of LPS, such as that derived from intestinal translocation or a low-grade infection, may induce severe illness without overt signs of infection.

The increased mortality associated with TNF-α priming was also associated with increased [IFN-γ] and blood [lactate]. Mean peak serum [IFN-γ] and blood [lactate] values were significantly increased in the primed rats compared with unprimed rats after LPS challenge except for the low-priming and low-LPS challenge group. Comparison of these two measures revealed a correlation (r = 0.396), and we observed that death occurred more frequently when both measures were elevated. This is similar to findings in other studies (26, 29) that show a correlation between [IFN-γ] and [lactate] values with mortality.

The generalized Schwartzman reaction, another lethal shock model, is also thought to be mediated by IFN-γ. This model induces lethality using a subcutaneously injected priming dose of LPS before a venous injection of LPS. This shock model requires precise timing and concentrations of LPS to obtain lethality (17). Ozmen and co-workers (17) have demonstrated that the increased lethality of the generalized Schwartzman reaction is mediated by IFN-γ although they suggest that other LPS-induced factors are also involved.

Interestingly, our only measure that was not significantly increased after LPS challenge between the primed and unprimed animals was TNF-α. LPS did, however, induce a significant time- and dose-dependent increase in TNF-α in both primed and unprimed rats treated with LPS, which suggests that there was not a downregulation of the TNF-α response due to the TNF-α priming. Previous studies (11, 12) have demonstrated that a large bolus dose of TNF-α increases expression and release of soluble TNF-α receptors that possess the ability to neutralize serum TNF-α. Therefore, in our studies, TNF-α priming may increase expression and release of the soluble TNF-α receptor thereby inhibiting an enhanced TNF-α response to LPS challenge. This is further supported by our observations that rats primed with high-dose TNF-α that were then rechallenged 2 days later with the same dose of TNF-α demonstrated a sixfold decrease in mean peak serum concentrations at 1 min postinfusion from the priming to the challenge dose.

One consideration of this observed sixfold decrease in [TNF-α] after a second TNF-α challenge is that rats primed with high-dose TNF-α and then challenged with LPS may also exhibit a similar decrease in measurable TNF-α. Therefore, TNF-α, which may be up to six times greater than measured, may be a significant mediator of the pathological process leading to death in these animals. However, our data do not support this hypothesis because the mortality rate of rats challenged with high-dose LPS was similar whether the rats were primed with low TNF-α (25%) or high TNF-α (38%). Rats primed with low-dose TNF-α that were then challenged with high-dose TNF-α did not demonstrate the same sixfold decrease in TNF-α that was seen when high-dose TNF-α was used for both priming and challenge. In fact, the challenge dose of high TNF-α in rats primed with low-dose TNF-α resulted in the same [TNF-α] (175.3 ± 37.4 ng/ml). Therefore, the observed decrease in [TNF-α] of the high-dose TNF-α priming group does not appear to be related to mortality.

The finding that TNF-α priming did not alter the LPS-induced TNF-α response but did significantly enhance the LPS-induced IFN-γ response suggests that IFN-γ release is regulated independently from TNF-α release. However, because IFN-γ production is primarily controlled by the transcription factor IFN regulatory factor-1 (IRF-1) and TNF-α induces activation of IRF-1, we speculate that TNF-α priming may increase IRF-1 expression and result in increased IFN-γ release (3, 16, 22).

In conclusion, our study demonstrates that TNF-α primes the LPS-induced response in chronically catheterized rats. The results of this study are contradictory to previous studies in which animals were pre-treated with TNF-α 24 h before treatment with LPS. However, the results of our study are more clinically relevant for the following reasons: 1) TNF-α was administered 48 h before LPS administration to better correlate with the onset of MSOF after TNF-α-inducing noninfectious events, 2) a nonstressed animal model was used in which the LPS-induced inflammatory response was not attenuated, and 3) lower doses of TNF-α and LPS were administered. The results of this study may explain the timing and increased susceptibility of patients for the development of MSOF after severe trauma.

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