Inhibiting Interleukin-1 and Tumor Necrosis Factor-α Does Not Reduce Induction of Plasminogen Activator Inhibitor Type-1 by Endotoxin in Rats In Vivo

By J.J. Emeis, R. Hoekzema, and A.F. de Vos

In experimental animals and humans, intravenous (IV) injection of endotoxin induces large increases in circulating plasminogen activator inhibitor type-1 (PAI-1), a major inhibitor of blood fibrinolysis. A similar increase is seen after the injection of interleukin-1 (IL-1) or of tumor necrosis factor-α (TNF-α), suggesting that these cytokines mediate the induction, by endotoxin, of PAI-1. To test this hypothesis we pretreated rats, before IV endotoxin, with compounds that inhibit the formation of cytokines (pentoxifylline; dexamethasone), or with compounds that inhibit the action of these cytokines (anti-TNF antiserum for TNF-α; IL-1 receptor antagonist for IL-1). None of these pretreatments affected the induction of PAI-1 synthesis by endotoxin. However, pretreatment did reduce the endotoxin-induced increase in plasma tPA antigen concentration. Thus, the data suggest that, in rats in vivo, TNF-α and IL-1 are not significantly involved in the induction of PAI-1 by endotoxin.

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PLASMINOGEN ACTIVATOR inhibitor type-1 (PAI-1) is one of the plasma factors that determine the plasminogen activating potential of blood. Thus, increased blood levels of this inhibitor are likely to reduce blood fibrinolysis and thrombolysis, making PAI-1 a potential risk factor for cardiovascular disease and thromboembolism.1-3 Many types of insult will induce increased levels of plasma PAI-1 activity, examples of which are sepsis, surgery, and trauma.4,5 Gram-negative endotoxemia, both experimentally6,7 and clinically,8,14 strongly increases the plasma level of PAI-1 in humans.

In previous studies, we17-20 and others21-23 have shown that the injection of low doses of endotoxin into experimental animals will induce large and rapid increases of circulating PAI-1, due to de novo synthesis of PAI-1.24 As a corollary to these studies we have shown that the endotoxin-induced cytokines interleukin-1α (IL-1α),25 IL-1β,26 and tumor necrosis factor-α (TNF-α)27 will also increase plasma PAI activity in rats.

In combination, these observations suggested to us that the endotoxin-induced increase in PAI-1 activity might be mediated by endotoxin-induced cytokines, especially IL-1 and TNF-α. To test this hypothesis we pretreated rats either with compounds that prevent the synthesis and/or secretion of cytokines (dexamethasone, pentoxifylline) or with compounds that inhibit the effects of cytokines (IL-1 receptor antagonist for IL-1; anti-TNF antiserum for TNF-α), and measured the subsequent endotoxin-induced increase in PAI. We also measured tissue-type plasminogen activator (tPA) antigen levels in these rats, because tPA is known to be increased as well after endotoxin injection. The increase in PAI activity was not affected by any of these treatments, whereas the increase in tPA antigen was. We propose that, contrary to our hypothesis, the endotoxin-induced cytokines IL-1 and TNF-α are not significantly involved in the induction of PAI-1 by endotoxin.

MATERIALS AND METHODS

Materials. Endotoxin (lipopolysaccharide [LPS], from Escherichia coli serotype 0128:B12; Sigma, St Louis, MO) was dissolved in sterile saline to a concentration of 10 μg/mL. All other compounds were dissolved in sterile saline containing 1% (wt/vol) sterile, pyrogen-free human serum albumin (Central Laboratory of the Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). Recombinant human TNF-α (2.5 × 106 U/mg protein; a gift from Dr W. Piers, Biogent, Gent, Belgium) was diluted to 10 μg/mL. Recombinant human IL-1β (106 U/mg; a gift from Dr S. Gillis, Immunex, Seattle, WA) was diluted to 2 μg/mL. Recombinant mouse TNF-α, rabbit-antimouse TNF-α antiserum, and an enzyme-linked immunosorbent assay (ELISA) kit for mouse TNF-α were obtained from Genzyme (Boston, MA). Recombinant human IL-1 receptor antagonist (IL-1ra; a gift from Dr D.E. Tracey, The Upjohn Co, Kalamazoo, MI) was diluted to 1 mg/mL. Pentoxifylline (Trental) was from Hoechst AG (Wiesbaden, Germany), and was used as supplied (20 mg/mL). Dexamethasone (Sigma) was first dissolved in ethanol (10 mg/mL), and then diluted to 1 mg/mL in saline. Recombinant human tissue-type plasminogen activator (tPA; Activase) was from Genentech (San Francisco, CA). Recombinant rat and mouse IL-1α, IL-1β, and IL-1ra have been described previously.27 Rabbit-antirat PAI-1 IgG was obtained from American Diagnostica (Greenwich, CT). Biotine, avidin-peroxidase, and tetramethylbenzidine were from Pierce (Rockford, IL).

Animal experimentation. Male Wistar rats (200 to 250 g body weight) were obtained from the Broekman Institute (Someret, The Netherlands). All experiments were performed under Nembutal anesthesia (60 mg/kg, intraperitoneally). Injections were administered into the vein of the penis. Blood was obtained by aortic puncture into precooled syringes, and anticoagulated with 0.13 mol/L trisodium citrate (1 vol to 9 vol of blood). Platelet-poor plasma was immediately prepared at 4°C by centrifugation for 10 minutes at 2,000g, and stored at −20°C. Serum was prepared for 30 minutes at 37°C and 30 minutes at 4°C, followed by centrifugation for 10 minutes at 2,000g.

Animal experiments had been approved by the Animal Experiments Committee of The Netherlands Organization for Applied Scientific Research TNO, and were in accordance with the guidelines on animal experimentation presented to the International Committee of Thrombosis and Haemostasis.28

Experimental design. Rats were injected with a compound under study, followed—after an interval of 30 to 60 minutes, as detailed...
Table 1. Effect of Pretreatment With Pentoxifylline or Dexamethasone on the Induction of TNF-α by Endotoxin

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>TNF-α (pg/mL)</th>
<th>Geometric Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Individual Data</td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>45,640</td>
<td>28,958</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>19,281</td>
<td>11,486</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1,430</td>
<td>992</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>1,094</td>
<td>307</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>278</td>
<td>248</td>
</tr>
</tbody>
</table>

Rats (four per group) were pretreated with pentoxifylline (50 mg/kg IV, 1 hour before LPS), or with dexamethasone (2 mg/kg intraperitoneally, 2 hours before LPS), and subsequently injected with LPS (10 μg/kg). Serum for the determination of TNF-α concentrations was obtained 1 hour after LPS injection. For the determination of TNF-α, see Materials and Methods.

below—by the intravenous (IV) injection of LPS (10 μg/kg), recombinant human TNF-α (10 μg/kg), or recombinant human IL-1β (1 μg/kg). Blood for the assay of PAI-1 activity was obtained 3 hours later. To assess the effects of pretreatment on the induction of TNF-α by LPS, rats were bled 1 hour after LPS.

**TNF-α assay.** TNF-α concentrations were determined in serum by a cytotoxicity assay, using a subclone of the WEHI 164/13 mouse fibrosarcoma cell line as the indicator cell, essentially as described by Espievik and Nissen-Meyer. The assay was calibrated using recombinant mouse TNF-α, and had a lower limit of sensitivity of 1 pg/mL. Rat serum samples were assayed at dilutions of 1:10, 25, and 1,250. In the serum samples, TNF-α was specifically detected at levels ≥50 pg/mL.

**PAI assay.** Plasma PAI concentrations were determined in appropriately diluted plasma by titration with human recombinant tPA (Activase), followed by determination of the residual tPA activity, as described by Verheijen et al. Results will be expressed as units (U) per milliliter, 1 U/mL being equivalent to 1 ng of tPA inhibited per mL of plasma.

**tPA antigen assay.** Rat tPA antigen was determined by an ELISA, as follows. Microtiter plates were coated with rabbit-antibody tPA IgG (4 μg/mL in carbonate buffer, pH = 9.0) overnight at 4°C, and washed. Samples (diluted in phosphate-buffered saline [PBS] containing 5 mg/mL casein) were incubated overnight at 4°C and washed. Bound tPA was subsequently quantitated using biotinylated rabbit-antibody tPA IgG, followed by avidin-peroxidase and tetramethylbenzidine. Rat tPA purified from Lz cells (0.5 to 4.0 ng/mL) was used as standard. The rat Lz tPA had been calibrated against recombinant rat JMI-229 tPA. Results will be expressed as nanograms of rat tPA per milliliter.

**Statistics.** Statistical significance of differences between groups will be analysed by Student’s t-test, or by one-way ANOVA followed by Bonferroni’s modified t-test, as indicated. Differences will be considered significant if P (two-sided) < .05.

**RESULTS**

**Effect of pentoxifylline and dexamethasone on TNF-α induction.** A pilot experiment (not shown) demonstrated that peak TNF-α levels were found 1 hour after the injection of LPS at a dose of 10 μg/kg. No TNF-α was detectable in saline-injected controls. Pretreatment of rats with pentoxifylline (50 mg/kg IV 1 hour before LPS), or with dexamethasone (2 mg/kg intraperitoneally 2 hours before LPS), significantly reduced serum TNF-α levels (measured 1 hour after LPS) from a mean value of 20,500 pg/mL in saline-pretreated rats to 770 pg/mL (4% of controls) in pentoxifylline-pretreated rats, and to 339 pg/mL (2% of controls) in dexamethasone-pretreated rats (Table 1). At 3 hours after LPS, mean (n = 3) serum TNF-α values were 132 pg/mL in saline-pretreated rats and 133 pg/mL in pentoxifylline-pretreated rats, whereas no TNF-α was detected in dexamethasone-pretreated animals. Closely similar values were found when TNF-α levels were determined by ELISA, using an ELISA kit for mouse TNF-α which also measures rat TNF-α (data not shown).

**Effect of LPS on plasma PAI activity.** LPS (10 μg/kg) induced, as we described previously, a large increase in plasma PAI activity. In the present study, the plasma PAI activity averaged, in all saline-pretreated animals combined, 121 U/mL at 3 hours after LPS injection, representing a 25-fold increase over the baseline level of 4 to 6 U/mL. The identification of the increased inhibitory activity as PAI-1 activity has been described, and could in the present study be confirmed by quenching the LPS-induced activity by rabbit-antibody PAI-1 IgG (not shown).

**Effect of pentoxifylline and dexamethasone on PAI induction by LPS.** Pretreatment with pentoxifylline (20, 50, or 100 mg/kg IV, 1 hour before LPS) had no effect on the induction of PAI by LPS. Saline-pretreated animals had PAI levels of 134 ± 33 U/mL (mean ± SD; n = 9); pentoxifylline-pretreated rats had PAI levels of 118 ± 18 U/mL for pentoxifylline at 20 mg/kg (n = 5); 123 ± 13 U/mL for pentoxifylline at 50 mg/kg (n = 4); and 111 ± 21 U/mL for pentoxifylline at 100 mg/kg (n = 4). These differences were not significant by one-way ANOVA. Injection of only pentoxifylline did not affect plasma PAI activity, as we described previously. Acute pretreatment with dexamethasone (2 mg/kg intraperitoneally 2 hours before LPS) had no effect on the induction of PAI by LPS (Table 2), nor did subchronic pretreatment with dexamethasone (2 mg/kg intraperitoneally, once daily for 4 days) (Table 2). Corticosterone had no effect either (Table 2). Dexamethasone alone (without LPS) increased PAI activity about twofold.

Table 2. Effect of Dexamethasone and Corticosterone on the Induction of Plasma PAI Activity by Endotoxin

<table>
<thead>
<tr>
<th>PAI Activity (U/mL)</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol controls</td>
<td>111 ± 28</td>
</tr>
<tr>
<td>Dexamethasone*</td>
<td>102 ± 24</td>
</tr>
<tr>
<td>Ethanol controls</td>
<td>133 ± 11</td>
</tr>
<tr>
<td>Dexamethasone†</td>
<td>97 ± 24</td>
</tr>
<tr>
<td>Ethanol controls</td>
<td>105 ± 11</td>
</tr>
<tr>
<td>Corticosterone‡</td>
<td>112 ± 16</td>
</tr>
</tbody>
</table>

All data shown are mean ± SD. Rats were pretreated with corticosteroids as indicated, followed by LPS (10 μg/kg IV). Plasma for PAI determinations was obtained 3 hours after LPS. For further details, see Materials and Methods. No significant differences between treatment and control groups were present.

* Dexamethasone 2 mg/kg intraperitoneally, daily for 4 days before LPS (10 μg/kg IV).
† Dexamethasone 2 mg/kg intraperitoneally, 2 hours before LPS.
‡ Corticosterone 50 mg/kg intraperitoneally, 2 hours before LPS.
Table 3. Effect of Anti-TNF Antiserum, Interleukin-1-Receptor Antagonist, or Both, on PAI Induction

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>PAI Activity (U/mL)</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline, then LPS</td>
<td>131 ± 45</td>
<td>10</td>
</tr>
<tr>
<td>2. Control rabbit serum, then LPS</td>
<td>145 ± 5</td>
<td>4</td>
</tr>
<tr>
<td>3a. Anti-TNF antiserum (0.1 mL), then LPS</td>
<td>136 ± 57</td>
<td>4</td>
</tr>
<tr>
<td>3b. Anti-TNF antiserum (0.3 mL), then LPS</td>
<td>113; 141</td>
<td>2</td>
</tr>
<tr>
<td>4a. IL-1ra (1 mg/kg), then LPS</td>
<td>154 ± 32</td>
<td>4</td>
</tr>
<tr>
<td>4b. IL-1ra (4 mg/kg), then LPS</td>
<td>133; 148</td>
<td>2</td>
</tr>
<tr>
<td>5. Anti-TNF (0.1 mL) plus IL-1ra</td>
<td>156 ± 8</td>
<td>4</td>
</tr>
<tr>
<td>6. Control rabbit serum, then TNF</td>
<td>52 ± 7</td>
<td>4</td>
</tr>
<tr>
<td>7a. Anti-TNF antiserum (0.1 mL), then TNF</td>
<td>30 ± 4</td>
<td>4</td>
</tr>
<tr>
<td>7b. Anti-TNF antiserum (0.3 mL), then TNF</td>
<td>5; 8</td>
<td>2</td>
</tr>
<tr>
<td>8. Saline, then IL-1</td>
<td>16 ± 4</td>
<td>4</td>
</tr>
<tr>
<td>9a. IL-1ra (1 mg/kg), then IL-1</td>
<td>10 ± 1</td>
<td>4</td>
</tr>
<tr>
<td>9b. IL-1ra (4 mg/kg), then IL-1</td>
<td>5; 6</td>
<td>2</td>
</tr>
</tbody>
</table>

All data shown are mean ± SD, or individual values of two animals.

Effect of anti-TNF antiserum, IL-1 receptor antagonist (IL-1ra), or both, on PAI induction by LPS. Pretreatment of rats with an antimouse-TNF antiserum (0.1 mL/rat) had no significant effect on the induction of PAI by LPS, compared to pretreatment with control rabbit serum (Table 3). Higher doses of the antiserum (0.15 or 0.3 mL/rat, n = 2 per dose) had no effect either (Table 3). The anti-TNF antiserum did reduce, though, the induction of PAI activity by recombinant human TNF-α, inhibiting partially at a dose of 0.1 mL/rat and fully at a dose of 0.3 mL/rat (Table 3). At a dose of 1 mg/kg, recombinant human IL-1ra significantly inhibited the induction of PAI-1 activity by recombinant human IL-1β (1 μg/kg), whereas at a dose of 4 mg/kg the receptor antagonist fully inhibited the induction by IL-1β (Table 3). In contrast, IL-1ra did not affect the induction of PAI by LPS at all (Table 3). Combined pretreatment with anti-TNF and IL-1ra had no effect on the induction of PAI by LPS either (Table 3).

Additive effects of TNF-α and IL-1β. To see whether residual circulating levels of one cytokine would potentiate the other, rats (two per group) were injected with TNF-α (10 μg/kg) with or without simultaneous injection of a small dose of IL-1β (0.1 μg/kg), or with only IL-1β (0.1 μg/kg). No potentiating effect on PAI induction was observed: TNF-α alone gave PAI activities of 18 and 20 U/mL; IL-1β alone 8 and 11 U/mL; TNF-α plus IL-1β 19 and 20 U/mL. Similarly, no potentiation of IL-1β (1 μg/kg) by a small dose of TNF-α (1 μg/kg) was seen: IL-1β alone gave PAI activities of 25 and 25 U/mL; TNF-α alone 12 and 13 U/mL; IL-1β plus TNF-α 30 and 38 U/mL. IL-1β (1 μg/kg) plus TNF-α (10 μg/kg) gave 35 and 42 U/mL. These data suggest that the combined effect of IL-1β and TNF-α is additive rather than synergistic. This makes it unlikely that residual amounts of TNF-α activity (remaining after anti-TNF treatment) would potentiate IL-1β, or that residual IL-1β activity (after IL-1ra treatment) would potentiate TNF-α. The effect of TNF-α on PAI-1 was linearly related to the injected dose of TNF-α over the dose-range 0.5 to 60 μg/kg (n = 9; r = .895; P < .01).

Changes in tPA antigen concentrations. LPS induced a time-dependent increase in tPA antigen (Fig 1), preceding the induction of PAI which commences only after 1 hour.17 Increases in tPA antigen of a magnitude similar to that induced by LPS were found at 3 hours after the injection of IL-1β and TNF-α (Table 4). The tPA increase after LPS was partly inhibited by pentoxifylline, dexamethasone, anti-TNF antiserum, and IL-1ra (Table 4). Similarly, anti-TNF antiserum and IL-1-ra partly inhibited the tPA increase induced by, respectively, TNF-α and IL-1β (Table 4). Inhibition was not complete, presumably because the high PAI levels in the circulation caused the formation of more tPA-PAI complexes, which are cleared from the circulation more slowly than uncomplexed tPA.32

DISCUSSION

This study was designed to test the hypothesis that the induction of PAI-1 by endotoxin is mediated by the cytokines TNF-α and/or IL-1. This hypothesis originated from two sets of observations. Firstly, the observation that endotoxin, a potent inducer of PAI-1, is also a potent inducer in vivo of IL-1 and TNF-α. Secondly, the observation that both cytokines will increase PAI-1 in vivo. IL-1ra and β have been shown to induce PAI-1 in rats,17 whereas TNF-α
Table 4. Effect of Pretreatment on Endotoxin-Induced tPA Antigen Levels

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>tPA Antigen (ng/mL)</th>
<th>No. of Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline-treated control rats</td>
<td>2.4 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>2. Saline, then LPS</td>
<td>8.1 ± 2.5</td>
<td>10</td>
</tr>
<tr>
<td>3. Normal rabbit serum, then LPS</td>
<td>8.4 ± 3.0</td>
<td>4</td>
</tr>
<tr>
<td>4. Pentoxifylline, then LPS</td>
<td>6.2 ± 1.5</td>
<td>4</td>
</tr>
<tr>
<td>5. Dexamethasone, then LPS</td>
<td>6.3 ± 1.2</td>
<td>4</td>
</tr>
<tr>
<td>6. Anti-TNF antiserum, then LPS</td>
<td>6.3 ± 1.3</td>
<td>4</td>
</tr>
<tr>
<td>7. IL-1ra, then LPS</td>
<td>5.8 ± 1.6</td>
<td>4</td>
</tr>
<tr>
<td>8. Control rabbit serum, then TNF</td>
<td>5.7 ± 0.3</td>
<td>4</td>
</tr>
<tr>
<td>9. Anti-TNF antiserum, then TNF</td>
<td>3.2 ± 0.1</td>
<td>4</td>
</tr>
<tr>
<td>10. Saline, then IL-1</td>
<td>6.2 ± 1.0</td>
<td>3</td>
</tr>
<tr>
<td>11. IL-1ra, then IL-1</td>
<td>3.6 ± 0.3</td>
<td>3</td>
</tr>
</tbody>
</table>

All data shown are mean ± SD. Groups 2-7 were not significantly different by one-way ANOVA. When groups 2 and 3 and groups 4-7 were combined, the two new groups (non-pretreated vs pretreated) were significantly different by Student’s t-test (P < 0.1). Group 8 was significantly different from group 9, and group 15 from group 11, by Student’s t-test (P < 0.05). Rats were pretreated with saline, control rabbit serum (0.1 mL/rat), pentoxifylline (50 mg/kg IV), dexamethasone (2 mg/kg intraperitoneally), anti-TNF antiserum (0.1 mL/ rat), or IL-1ra (1 mg/kg), followed by either LPS (10 μg/kg), recombinant human TNF-α (10 μg/kg), or recombinant human IL-1β (1 μg/kg). Rats were killed 3 hours after LPS, TNF, or IL-1. For further details, see Materials and Methods.

Table 4 shows that pretreatment with various combinations of the two cytokines, but rather additive effects. Moreover, because the PAI response to TNF-α was linear over the TNF concentration range 0.5 to 60 μg/kg, we consider it unlikely that residual amounts of TNF (or IL-1) would significantly have affected the PAI concentrations.

In a previous publication we have shown that a variety of autacoids that are induced or released by endotoxin in vivo (e.g., cyclo-oxygenase and lipoxygenase products, platelet-activating factor, catecholamines, histamine, cyclic nucleotides, opioids, vasopressin, thrombin) are not involved in the induction of IL-1β. To block the effects of IL-1, we used an IL-1 receptor antagonist that effectively inhibits IL-1-induced effects in a variety of species, including rats. However, like dexamethasone, IL-1ra had no effect on PAI-1 induction by endotoxin in this study, though it inhibited the induction of PAI-1 by human IL-1β (Table 3).

Endotoxin not only induces increased plasma levels of PAI-1, but also of tPA, induction of tPA preceding that of PAI-1, and as has been observed both in humans and chimpanzees. Increased tPA antigen levels have also been described in humans after treatment with TNF-α. In the present study similar increases were noted after treatment of rats with endotoxin (Fig 1), TNF-α, and IL-1β (Table 4), an observation not made in rats before. The increase in tPA antigen after the injection of TNF-α was reduced by anti-TNF antiserum, and the increase after IL-1β by IL-1ra (Table 4). The increase in tPA antigen after endotoxin injection was reduced, though to a lesser extent, by pretreatment with pentoxifylline, dexamethasone, anti-TNF antiserum, and IL-1ra. This suggests that pretreatment with anti-TNF antiserum and IL-1ra was not only effective against injected (human) TNF-α or IL-1β, but also against endogenous TNF-α and IL-1. That tPA was not reduced to normal control levels is presumably due to the fact, mentioned above, that the induction of PAI-1 still occurred in all animals, resulting in increased circulating levels of tPA-PAI complexes that are detected by our tPA ELISA assay. Also, it is likely that tPA release is induced after endotoxin injection by other endotoxin-induced compounds such as platelet-activating factor, catecholamines, vasopressin, etc.

Because synergistic effects between TNF-α and IL-1 have been reported (eg, ref 56), and because in our experiments low residual levels of cytokines activity are likely to be present during treatment with antisera or with receptor antagonist, we investigated whether synergistic effects on PAI synthesis could be detected. As described above, no such synergy between TNF-α and IL-1β was found, using various combinations of the two cytokines, but rather additive effects. Moreover, because the PAI response to TNF-α was linear over the TNF concentration range 0.5 to 60 μg/kg, we consider it unlikely that residual amounts of TNF (or IL-1) would significantly have affected the PAI concentrations.

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Endotoxin not only induces increased plasma levels of PAI-1, but also of tPA, induction of tPA preceding that of PAI-1, and as has been observed both in humans and chimpanzees. Increased tPA antigen levels have also been described in humans after treatment with TNF-α. In the present study similar increases were noted after treatment of rats with endotoxin (Fig 1), TNF-α, and IL-1β (Table 4), an observation not made in rats before. The increase in tPA antigen after the injection of TNF-α was reduced by anti-TNF antiserum, and the increase after IL-1β by IL-1ra (Table 4). The increase in tPA antigen after endotoxin injection was reduced, though to a lesser extent, by pretreatment with pentoxifylline, dexamethasone, anti-TNF antiserum, and IL-1ra. This suggests that pretreatment with anti-TNF antiserum and IL-1ra was not only effective against injected (human) TNF-α or IL-1β, but also against endogenous TNF-α and IL-1. That tPA was not reduced to normal control levels is presumably due to the fact, mentioned above, that the induction of PAI-1 still occurred in all animals, resulting in increased circulating levels of tPA-PAI complexes that are detected by our tPA ELISA assay. Also, it is likely that tPA release is induced after endotoxin injection by other endotoxin-induced compounds such as platelet-activating factor, catecholamines, vasopressin, etc.

Because synergistic effects between TNF-α and IL-1 have been reported (eg, ref 56), and because in our experiments low residual levels of cytokines activity are likely to be present during treatment with antisera or with receptor antagonist, we investigated whether synergistic effects on PAI synthesis could be detected. As described above, no such synergy between TNF-α and IL-1β was found, using various combinations of the two cytokines, but rather additive effects. Moreover, because the PAI response to TNF-α was linear over the TNF concentration range 0.5 to 60 μg/kg, we consider it unlikely that residual amounts of TNF (or IL-1) would significantly have affected the PAI concentrations.
in rats, inhibit the profibrinolytic, tPA-mediated, effect of endotoxin without interfering with its antifibrinolytic, PAI-1-mediated effect. In combination, these effects might then shift the fibrinolytic balance in blood even further to an inhibitory state, worsening the procoagulant effects of endotoxin. It would be of interest to study whether this situation, as described here for rats, also applies to primates and humans.

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