The vascular endothelium plays an important role in fibrinolysis by producing tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI). The monokine tumor necrosis factor (human recombinant TNF) increased the production of PAI by cultured human endothelial cells from umbilical vein (twofold) and from foreskin microvessels (four to eight fold). This was demonstrated by titration of endothelial cell-conditioned medium with t-PA, by reverse fibrin autography, and by immunoprecipitation of [35S]PAI-1 by anti-PAI-1 IgG. TNF also induced a marked increase of PAI-1 messenger RNA (mRNA) in the cells. The stimulation of PAI activity by TNF was seen at 4 U/mL and reached a maximum at 500 U/mL. Human recombinant lymphotixin and interleukin-1 (α and β) also stimulated the production of PAI activity, while interleukin-6 was ineffective. Separate additions of TNF or interleukin-1 (IL-1) at optimal concentrations (500 U/mL and 5 U/mL, respectively) resulted in a comparable stimulation of PAI production by endothelial cells. The simultaneous addition of both mediators resulted in an additive effect. The effect of TNF could not be prevented by the addition of polymyxin B or by anti-IL-1 antibodies. Therefore, it is unlikely that TNF acts through the induction of IL-1 secretion by endothelial cells. Two hours after a bolus injection of 250,000 U/kg TNF into rats, a fivefold increase in circulating PAI levels was found. In the next ten hours, the levels returned to normal. Blood platelets do not significantly contribute to the increase in circulating PAI, because the number of platelets did not change after TNF injection and the amount of PAI in blood platelets is not sufficient for several hours during an increase in PAI activity. The acute phase reactants, fibrinogen and α2-antiplasmin in rat plasma, were altered little if any two to 24 hours after injection of 250,000 U/kg TNF. In vitro, TNF did not change PAI production by human and rat hepatocytes in primary monolayer culture. Therefore, it is most likely that vascular endothelial cells contribute to the increased amount of circulating PAI induced by TNF in vivo. This increase in PAI activity might decrease fibrinolysis.

**Tumor Necrosis Factor Increases the Production of Plasminogen Activator Inhibitor in Human Endothelial Cells In Vitro and in Rats In Vivo**

By Victor W.M. van Hinsbergh, Teake Kooistra, Eva A. van den Berg, Hans M.G. Princen, Walter Fiers, and Jef J. Emeis

The removal of a temporary fibrin matrix in the circulatory system depends mainly on the action of the protease plasmin, which, on a fibrin clot, is generated from itszymogen by tissue-type plasminogen activator (t-PA). The plasminogen activator (PA) rapidly interacts with a plasminogen activator inhibitor (PAI), PAI-1, and PAI-1 is synthesized by various cultured cells, including endothelial cells, and human hepatocytes, and it is present in freshly isolated blood platelets. Several clinical studies have suggested a role of PAI-1 in deep venous thrombosis and an association between postoperative hyperresponse in PA inhibition and deep venous thrombosis. In myocardial infarction patients, an increase in the plasma level of PAI-1 has been reported. We have observed that tumor necrosis factor (TNF) increases the production of PAI-1 activity by endothelial cells in vitro and in rats. Besides IL-1, activated macrophages can produce large amounts of another mediator of acute phase response, tumor necrosis factor (TNF), and TNF has many effects similar to those of IL-1, including the induction of tissue factor, and leukocyte adhesion molecules on endothelial cells. TNF, which is identical to cachectin, can induce hypertriglyceridemia. An occasional coincidence between hypertriglyceridemia and decreased fibrinolysis has been reported. We hypothesized that in certain patients a common mediator may underlie the hypertriglyceridemia and the decreased fibrinolysis, and we have investigated whether TNF influences the production of PAI.

**MATERIALS AND METHODS**

*Materials.* Human recombinant TNF and lymphotixin (expressed in Chinese hamster ovary (CHO) cells) were gifts of Dr Jan Tavernier (Biogent, Gent, Belgium). The TNF preparation contained 2.45 × 10^6 U/mg protein and <40 ng lipopolysaccharide (LPS) per milligram protein; the specific activity of lymphotixin was 1.6 × 10^6 U/mg protein. Human recombinant interleukin-6 (expressed in yeast cells) was prepared in the Laboratory of Molecular Biology, Gent, Belgium; it was biologically active and had a specific activity of 1.4 × 10^6 U/mg protein. Human recombinant IL-1α (pI 5) and IL-1β (pI 7) and rabbit anti-IL-1 antibodies were purchased from Genzyme (Haverhill, UK); polymyxin B was purchased from Sigma Chemical Corp (St Louis). Rabbit anti-PAI-1 IgG were raised in our laboratory. The PAI-1 messenger RNA (mRNA) probe was a partial (1,400-bp) cDNA copy of the human PAI-1 mRNA. Other materials used have been described previously.

**Animal experiments.** Male Wistar rats (200 to 300 g body weight) were used under Nembutal anesthesia (60 mg/kg, administered intraperitoneally). TNF was injected via the vein of the penis in a volume of 0.5 mL/rat. Control rats received the vehicle only, or TNF (10 µg/kg) heated for 15 minutes at 90°C. Blood was obtained by aortic puncture and anticoagulated with citrate (final concentration 0.013 mol/L). Platelet-free plasma was prepared and stored at −20°C. Blood leukocytes, platelets, and microhematocrit were determined by standard procedures. Plasma levels of fibrinogen and α2-macroglobulin were determined by radial immunodiffusion, using the vascular endothelium plays an important role in fibrinolysis by producing tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI). The monokine tumor necrosis factor (human recombinant TNF) increased the production of PAI by cultured human endothelial cells from umbilical vein (twofold) and from foreskin microvessles (four to eight fold). This was demonstrated by titration of endothelial cell-conditioned medium with t-PA, by reverse fibrin autography, and by immunoprecipitation of [35S]PAI-1 by anti-PAI-1 IgG. TNF also induced a marked increase of PAI-1 messenger RNA (mRNA) in the cells. The stimulation of PAI activity by TNF was seen at 4 U/mL and reached a maximum at 500 U/mL. Human recombinant lymphotixin and interleukin-1 (α and β) also stimulated the production of PAI activity, while interleukin-6 was ineffective. Separate additions of TNF or interleukin-1 (IL-1) at optimal concentrations (500 U/mL and 5 U/mL, respectively) resulted in a comparable stimulation of PAI production by endothelial cells. The simultaneous addition of both mediators resulted in an additive effect. The effect of TNF could not be prevented by the addition of polymyxin B or by anti-IL-1 antibodies. Therefore, it is unlikely that TNF acts through the induction of IL-1 secretion by endothelial cells. Two hours after a bolus injection of 250,000 U/kg TNF into rats, a fivefold increase in circulating PAI levels was found. In the next ten hours, the levels returned to normal. Blood platelets do not significantly contribute to the increase in circulating PAI, because the number of platelets did not change after TNF injection and the amount of PAI in blood platelets is not sufficient for several hours during an increase in PAI activity. The acute phase reactants, fibrinogen and α2-antiplasmin in rat plasma, were altered little if any two to 24 hours after injection of 250,000 U/kg TNF. In vitro, TNF did not change PAI production by human and rat hepatocytes in primary monolayer culture. Therefore, it is most likely that vascular endothelial cells contribute to the increased amount of circulating PAI induced by TNF in vivo. This increase in PAI activity might decrease fibrinolysis.

**Animal experiments.** Male Wistar rats (200 to 300 g body weight) were used under Nembutal anesthesia (60 mg/kg, administered intraperitoneally). TNF was injected via the vein of the penis in a volume of 0.5 mL/rat. Control rats received the vehicle only, or TNF (10 µg/kg) heated for 15 minutes at 90°C. Blood was obtained by aortic puncture and anticoagulated with citrate (final concentration 0.013 mol/L). Platelet-free plasma was prepared and stored at −20°C. Blood leukocytes, platelets, and microhematocrit were determined by standard procedures. Plasma levels of fibrinogen and α2-macroglobulin were determined by radial immunodiffusion, using...
Fig 1. Effect of TNF on PAI production by human umbilical artery endothelial cells. (A) Endothelial cells were incubated for various time periods at 37°C in M199 medium and 10% human serum with 500 U/mL TNF ( ), 10 μg/mL bacterial LPS ( ), or without addition ( ). (B) Cells were incubated for six hours in M199 medium and 10% human serum ( ) or in M199 medium and 0.03% pyrogen-free albumin ( ) with various concentrations of TNF. PAI activity in the supernatant fluid was assayed in titration of t-PA in a fixed volume of conditioned medium as indicated under Materials and Methods. (Mean ± range of two representative cultures.)

monospecific antiserum against rat fibrinogen and rat α2-macroglobulin.25 Plasminogen and α2-antiplasmin were determined spectrophotometrically.25

Cell experiments. Endothelial cells from human umbilical artery and vein and from human foreskin microvessels were isolated, cultured, and characterized as previously described.24,27 Endothelial cell-conditioned media (ECCM) were obtained by incubating confluent cells at 37°C for the indicated period of time in M199 medium supplemented with 10% human serum, 20 mmol/L HEPES, and penicillin and streptomycin. The ECCM were immediately centrifuged and stored at −20°C until use. Serum-free ECCM were prepared by incubating the cells in M199 medium and 0.03% pyrogen-free human serum albumin, 20 mmol/L HEPES, and penicillin and streptomycin.

Human and rat hepatocytes were isolated and cultured essentially as previously described.29 The cells were incubated for various time periods in Williams’ E medium supplemented with 10% fetal bovine serum, 135 mmol/L insulin, and 1 μmol/L dexamethasone in the presence and absence of various concentrations of TNF.

Assay of t-PA and PA inhibitor. Plasminogen activator activity in the ECCM was detected by a fibrin autography technique25 after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8% gels) of ECCM.29 The t-PA antigen levels were assayed by enzyme immunoassay.21

PAI activity was measured by titration of t-PA into a fixed volume of ECCM or control medium (usually 5 or 10 μL).32 The titration curves were determined in triplicate (intra-assay variation was <5%). Reverse fibrin autography was performed essentially by the method of Loskutoff et al4 with t-PA as lysis inducing agent.

Immunoprecipitation of radiolabeled PAI-1 from [35S]-methionine-labeled ECCM was performed as previously described.27

RNA isolation and assay of PAI-1 mRNA. Total RNA was isolated from cultured endothelial cells as described by Lizardi et al23 with some minor modifications.29 Gel electrophoresis, Northern blotting, and hybridization were performed according to Maniatis et al. The PAI-1 probe used was a partial (1,400 bp) cDNA copy of the human PAI-1 mRNA and was radiolabeled by nick translation to approximately 10^7 cpm 32P/μg DNA.24

RESULTS

Effect of TNF on the production of PAI-1 by human umbilical artery and vein endothelial cells. Endothelial cells cultured from human umbilical artery and vein produce marked amounts of PAI-1 in their supernatant medium (Fig 1A). The addition of TNF (human recombinant TNF-α) resulted in a 1.5 to three fold increase in production of PAI.
activity. This increase was detectable two hours after TNF addition and lasted for at least 24 hours. The stimulation of PAI production was observed with 4 U/mL TNF and reached a maximum at 500 U/mL TNF (Fig 1B, closed symbols). At concentrations higher than 500 U/mL, TNF became cytotoxic for the cells, as observed by an increased number of detached cells after a 24-hour incubation period. The concomitant presence of human serum appeared to be necessary to obtain stimulation of PAI production by endothelial cells, because TNF was ineffective when the cells were incubated in medium supplemented with pyrogen-free human serum albumin only (Fig 1B, open symbols). The TNF-induced increase in PAI production by endothelial cells was further demonstrated by reverse fibrin autography (Fig 2A) and by immunoprecipitation of [35S]-methionine-labeled PAI by specific anti-PAI-1 IgG (Fig 2B). In contrast to PAI, the production of t-PA was not significantly influenced by TNF, as is shown by fibrin autography after SDS-PAGE of endothelial cell conditioned medium in Fig 2C. The effect of TNF on PAI production was not only found with subcultured endothelial cells, but also with primary cultures of human umbilical vein endothelial cells (Fig 3). The increase in PAI activity induced by TNF was in the same order of magnitude as that induced by IL-1α, IL-1β, or bacterial LPS. Human recombinant lymphotoxin, which has been reported to bind to the same receptor as TNF, also induces the production of PAI activity by endothelial cells (Fig 4). Human recombinant interleukin-6 (5 to 500 U/mL) did not affect the PAI production by human endothelial cells (not shown).

Effect of TNF on the production of PAI activity and t-PA antigen by human foreskin microvascular endothelial cells. In the postconfluent state, subcultured human foreskin microvascular endothelial cells produced small amounts of PAI activity (1.3 ± 0.9 IU/mL), which became increased 6.4 ± 2.2 fold during 24 hours of exposure to 500 U/mL TNF (7.0 ± 3.8; mean ± SD of seven experiments). Similar to umbilical artery and vein endothelial cells, TNF was maximally active at 500 U/mL and reached a half maximal activity at about 20 U/mL TNF (Fig 5). Low concentrations of TNF, which increase PAI production, did not or only slightly change the production of t-PA antigen by microvascular endothelial cells (Fig. 5). However, at high TNF concentrations a consistent increase in t-PA antigen was found. During a 24-hour exposure of microvascular endothelial cells to 500 U/mL TNF, t-PA production was increased.

Table 1. Effect of TNF and IL-1 on PAI Activity in Conditioned Media From Human Endothelial Cells

<table>
<thead>
<tr>
<th>Addition</th>
<th>FMVEC</th>
<th>HUAEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.6 ± 0.4 (3)</td>
<td>10.4 ± 2.9 (4)</td>
</tr>
<tr>
<td>+500 U/mL TNF</td>
<td>5.1 ± 3.7 (3)</td>
<td>19.5 ± 4.7 (4)</td>
</tr>
<tr>
<td>+10 μg/mL polymyxin B</td>
<td>0.7 ± 0.4 (3)</td>
<td>9.3 ± 2.1 (2)</td>
</tr>
<tr>
<td>+500 U/mL TNF + 10 μg/mL polymyxin B</td>
<td>5.1 ± 3.5 (3)</td>
<td>16.7 ± 3.9 (2)</td>
</tr>
<tr>
<td>+5 U/mL IL-1β</td>
<td>5.0 ± 3.5 (3)</td>
<td>ND</td>
</tr>
<tr>
<td>+5 U/mL IL-1β + 500 U/mL TNF</td>
<td>9.7 ± 6.8 (3)</td>
<td>ND</td>
</tr>
<tr>
<td>+5 U/mL IL-1α</td>
<td>ND</td>
<td>18.1 ± 3.9 (2)</td>
</tr>
<tr>
<td>+5 U/mL IL-1α + 500 U/mL TNF</td>
<td>ND</td>
<td>30.0 ± 7.0 (2)</td>
</tr>
<tr>
<td>+10 μg/mL LPS</td>
<td>6.6 ± 2.6 (3)</td>
<td>22.6 ± 3.0 (2)</td>
</tr>
<tr>
<td>+10 μg/mL LPS + 10 μg/mL polymyxin B</td>
<td>1.5 ± 0.8 (3)</td>
<td>11.7 ± 1.3 (2)</td>
</tr>
</tbody>
</table>

Human foreskin microvascular endothelial cells (FMVEC) or umbilical artery endothelial cells (HUAEC) were incubated with TNF or IL-1 for 24 hours. The values represent the mean ± SD (range) for the number of cultures given in parentheses.

Abbreviation: ND, not determined.
2.0 ± 0.7 fold (3.3 ± 0.8 ng/mL in controls vs 6.9 ± 4.0 ng in TNF-treated cells; mean ± SD; n = 4). This increase may be a consequence of changes in endothelial cell metabolism induced by TNF, rather than a direct action of TNF on the production of t-PA.

Comparison of the effect of TNF with those of bacterial LPS and IL-1. Similar to umbilical vein endothelial cells (Fig 3), the production of PAI activity by human microvascular endothelial cells was induced by LPS and IL-1 (Table 1). Addition of 10 μg/ml polymyxin B, which prevents the induction of PAI by 10 μg/mL LPS, did not prevent the increase in PAI by TNF (Table 1). This excludes a possible effect of LPS in our TNF preparation, which contained <1.6 ng LPS/10^6 U TNF as measured by Limulus assay. Furthermore, stimulation of PAI production by TNF and IL-1 was already visible after 2 hours, while LPS induced a PAI increase only after a lag period of 4 to 6 hours (Fig 1A). The induction of PAI activity by TNF was prevented by addition of actinomycin D (40 ng/mL) or cycloheximide (1 μg/mL) (not shown).

Because TNF can induce IL-1 production by endothelial cells,31,38 we compared the effects of IL-1 and TNF. Human recombinant IL-1α and IL-1β both stimulated the production of PAI activity in a concentration-dependent way, reaching a maximal stimulation at 1 to 5 U/mL. Although a similar maximal stimulation of PAI production was obtained with either 5 U/mL IL-1 or 500 U/mL TNF, addition of both agents together resulted in a twofold higher stimulation (Table 1). Furthermore, the addition of anti-IL-1 antibodies to the umbilical artery and microvascular endothelial cells did not affect the TNF-induced increase in PAI production, whereas their addition prevented the IL-1–induced increase.

**Table 2. Effect of TNF on the Production of PAI Activity by Human and Rat Hepatocytes**

<table>
<thead>
<tr>
<th>Human hepatocytes</th>
<th>2 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor 1, control</td>
<td>—</td>
<td>30 ± 1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>+ 20 U/mL TNF</td>
<td>—</td>
<td>30 ± 1</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>+ 500 U/mL TNF</td>
<td>—</td>
<td>28 ± 2</td>
<td>27 ± 2</td>
</tr>
</tbody>
</table>

| Donor 2, control  | 8 ± 1 | 17 ± 2 | 26 ± 4 |
| + 20 U/mL TNF     | 8 ± 0 | 16 ± 1 | 24 ± 1 |
| + 500 U/mL TNF    | 5 ± 0 | 14 ± 1 | 24 ± 1 |
| + 2,500 U/mL TNF  | 6 ± 1 | 18 ± 2 | 21 ± 3 |

<table>
<thead>
<tr>
<th>Rat hepatocytes (n = 3)</th>
<th>2 h</th>
<th>6 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 ± 2</td>
<td>6 ± 1</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>+ 500 U/mL TNF</td>
<td>3 ± 2</td>
<td>6 ± 1</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>+ 2,500 U/mL TNF</td>
<td>5 ± 3</td>
<td>6 ± 1</td>
<td>8 ± 1</td>
</tr>
</tbody>
</table>

**Table 6. Induction of PAI-1 mRNA by TNF in human umbilical artery (lanes 1, 2) and foreskin microvascular (lanes 3, 4) endothelial cells. Autoradiograph of [32P] PAI-1 cDNA hybridized to a Northern blot of RNA fractionated by formaldehyde-agarose gel electrophoresis. Total RNA preparations were made as described in Materials and Methods from endothelial cells that had been incubated for 6 hours in M199 medium and 10% serum with 500 U/mL TNF (lanes 2, 4) or without the addition (lanes 1, 3). Lanes 1 and 2 contain 2 μg of umbilical artery endothelial cell RNA; lanes 3 and 4 contain 1 μg of microvascular endothelial cell RNA.**
TNF increases PAI

![Graph showing PAI activity over time](image)

**Fig 7.** Effect of intravenous (IV) injection of TNF on PAI activity in rat plasma. (A) PAI activity was determined in rat plasma at various intervals after IV injection of 25,000 U/kg TNF (☆: 1 µg/kg), 250,000 U/kg TNF (○: 10 µg/kg), and 250,000 U/kg heated TNF (△). All data represent the mean ± SD of four rats per time point.

significantly increased after one hour, peaked fivefold after two hours, and subsequently returned to normal over the next ten hours. Injection of 25,000 U/kg TNF (1 µg/kg) resulted in a threefold increase of PAI activity after two hours (Fig 7). We have previously shown that an injection of bacterial LPS in the rat results in a marked increase in the PAI activity. A possible contamination of LPS was excluded by heating TNF, which destroys TNF activity but not LPS. Injection of heat-inactivated TNF (250,000 U/kg) did not change the plasma level of PAI activity (Fig 7). Because hematocrit and thrombocyte count did not change after injection of 250,000 U/kg TNF, it is unlikely that platelets contribute to the marked increase in circulating PAI activity. Total blood leukocyte counts decreased at one to three hours, followed by a slight increase at four to 24 hours.

It has been suggested that PAI behaves as a acute phase-responding protein. High doses of TNF indeed result in hypotension and shock. However, we found no or only a small response of classic acute phase proteins at the TNF doses that already maximally increased circulating PAI activity in the rat. Two hours after injection of 250,000 U/kg TNF, the plasma levels of plasminogen (118% ± 21% v 100% ± 12%), fibrinogen (82% ± 8% v 100% ± 17%), and α2-antiplasmin (104% ± 5% v 100% ± 11%) were unchanged (mean ± SD of four TNF-treated v four control rats). Twenty-four hours after TNF administration, the plasma levels of fibrinogen (124 ± 3 v 100 ± 17, P < .05), α2-antiplasmin (112 ± 2 v 100 ± 11, P < .05), and α2-macroglobulin were only slightly increased (four animals in each group).

**DISCUSSION**

This report shows that, as does IL-1, TNF induces an increase in PAI activity in the rat in vivo and an increase in the production of PAI-1 by cultured human umbilical cord vessel and microvascular endothelial cells. A four- to fivefold increase in PAI activity was found in rat plasma two hours after an injection of 1 or 10 µg TNF/kg. At high concentrations (200 to 600 µg TNF/kg), an injection of TNF in the rat induces hypotension and shock. Interestingly, the increase in PAI activity in vivo was seen at TNF concentrations that only marginally increased the acute phase reactants fibrinogen and α2-macroglobulin. The PAI activity in circulating plasma is immunologically identical to the PAI-1 produced by endothelial cells. The cellular origin of the basal level of plasma PAI-1 is still unknown. The increase in PAI activity in rat plasma after injection of TNF may be due to the release of PAI from blood platelets that contain PAI or to an increased generation of PAI activity by tissue cells, in particular endothelial cells and/or hepatocytes. However, the contribution of blood platelets to the TNF-induced increase in circulating PAI is insignificant because of the following factors:

1. We were unable to find any change in PAI release after the addition of 500 to 2,500 U/mL TNF to citrated platelet-rich rat plasma under conditions in which adenosine diphosphate (ADP) (5 µg/mL) and the calcium ionophore ionomycin (10 µmol/L) resulted in a 35% to 50% increase in PAI activity (Van Hinsbergh, unpublished).

2. The number of circulating platelets did not change during a two-hour period after injection of TNF in the rat.

3. Furthermore, even if the platelets released all their PAI activity, they would not significantly contribute to the plasma PAI level in the rat. The PAI activity in rat serum is 7.7 IU/mL as compared with 4.5 IU/mL in rat plasma (Emeis, unpublished). Given the rapid half-life time of PAI in the rat (3.5 minutes), the amount of PAI in circulating platelets is insufficient for several hours during an increase in PAI activity. Also, hepatocytes are not the probable source of the increase in circulating PAI activity. In vitro, TNF did not stimulate PAI production by human or rat hepatocytes. Furthermore, under our experimental conditions, TNF only marginally affected the production of acute phase proteins by the rat liver in vivo.

Although a contribution of other body cells cannot be excluded, it seems most likely that the vascular endothelial cells are involved in the increase in circulating PAI activity induced by TNF in vivo. If this were the case, the major contribution would be expected from the microvascular endothelial cells, which represent the major mass of endothelial cells in the body. Here, we have presented evidence that human microvascular endothelial cells indeed respond to TNF by a marked increase of their PAI production, and that this response is additive to an induction of PAI by IL-1. An additive effect of TNF and IL-1 was also demonstrated for the expression of tissue factor and leukocyte adhesion molecules on the surface of endothelial cells.

The concentration dependence of PAI induction of TNF in endothelial cells is the same as found for the expression of the membrane-bound proteins tissue factor and leukocyte adhesion molecule, and suggests a general receptor-mediated inductive phenomenon. Indeed, we found a marked increase in PAI-1 mRNA in all endothelial cell cultures after the addition of TNF. However, the mechanism of this induction is not clear. TNF receptors are present on endothelial cells, but no information is yet available about their signal transduction. The mechanism might be rather complicated, because—in contrast to the induction of tissue factor on endothelial cells—the TNF-induced increase in PAI activity depends on the presence of (an) additional serum factors.
factor(s). The requirement of serum does not merely reflect changes in the overall protein synthesis or cell viability, because (1) the \(^{35}\)S-methionine incorporation in endothelial proteins is only 1.5 to twofold higher in the presence of serum than in its absence,\(^\text{42}\) and (2) the addition of TNF to endothelial cells did not change or even slightly decreased their overall protein synthesis rate. To our present knowledge, PAI-1 is secreted constitutively, and a part of it accumulates in the extracellular matrix.\(^\text{43}\) Although PAI-1 can be released from the extracellular matrix of endothelial cells,\(^\text{43}\) the PAI production over an incubation period of several hours is mainly determined by the rate of synthesis of PAI-i.\(^\text{43}\) On the other hand, the increase in \(^{35}\)S-labeled PAI-1 antigen and in PAI-1 mRNA indicates that TNF acts on the synthesis of new PAI-1 molecules. Further experiments have to elucidate whether TNF acts mainly on the synthesis of PAI at the mRNA level, or whether posttranslational processing on PAI-1 is also changed with exposure of endothelial cells to TNF.

**ACKNOWLEDGMENT**

Human liver tissue was obtained through the Auxiliary Partial Liver Transplantation Program carried out at the Department of Surgery of the University Hospital Dijkzigt in Rotterdam, The Netherlands. Consent to use the remaining nontransplanted part of the liver for scientific research was given by the Medical Ethical Committee of the University Hospital Dijkzigt, Rotterdam.

We thank Dr J. Tavernier (Biogent) for providing us with human recombinant TNF and lymphotoxin, Drs B. Tison and Y. Guisez for the preparation of recombinant IL-6, and Dr D. Rijken for the assay of t-PA antigen. The technical assistance of R. van den Hoogen, M. Scheffer, D. Jense, J. van den Berg, P. Meijer, and A. Tons is gratefully acknowledged.

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Tumor necrosis factor increases the production of plasminogen activator inhibitor in human endothelial cells in vitro and in rats in vivo

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