Thrombosis Research (2008) **123**, 342–351

THROMBOSIS RESEARCH

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REGULAR ARTICLE

# Effects of IL-1β and IL-6 on tissue-type plasminogen activator expression in vascular endothelial cells

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Received 4 December 2007; received in revised form 22 February 2008; accepted 4 March 2008
Available online 27 May 2008

**KEYWORDS**
Fibrinolysis;
Inflammation;
Interleukin-1β;
Interleukin-6;
NF-κB;
t-PA

**Abstract**

*Introduction:* The increased risk of thrombus formation in inflammatory conditions is generally considered to be due to the pro-coagulant effect of inflammatory cytokines. However, cytokines may also decrease the expression of the key fibrinolytic enzyme tissue-type plasminogen activator (t-PA) causing a reduced clearance of emerging intravascular thrombi. This study investigated the effects of the inflammatory cytokines interleukin (IL)-1β and IL-6 on t-PA gene and protein expression, and elucidated by which signaling mechanisms the effects are mediated.
*Materials and Methods:* Cultured human umbilical vein endothelial cells (HUVEC) were exposed to recombinant IL-1β or IL-6. t-PA mRNA was quantified by real-time RT-PCR and t-PA antigen by ELISA. To clarify signaling mechanisms, selective inhibitors of major cytokine-activated signaling pathways were used. Interactions of nuclear proteins with potential t-PA gene regulatory elements were studied by gel shift assays.
*Results:* Already at low concentrations, IL-1β caused a distinct suppression of t-PA transcript and protein levels, mediated primarily by NF-κB signaling. This cytokine also increased binding of NF-κB subunits to a t-PA specific κB element. IL-6

*Abbreviations:* t-PA, Tissue-type plasminogen activator; IL, Interleukin; HUVEC, Human umbilical vein endothelial cells; NF-κB, Nuclear factor κB; MAPK, Mitogen activated protein kinase; PAI-1, Plasminogen activator inhibitor-1; TNF-α, Tumor necrosis factor-α; EMSA, Electrophoretic mobility shift assay; sIL-6R, Soluble interleukin-6 receptor.

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0049-3848/$ - see front matter © 2008 Published by Elsevier Ltd.
doi:10.1016/j.thromres.2008.03.013

stimulation *per se* did not affect t-PA mRNA or protein levels whereas soluble IL-6 receptor, in the presence of endogenous IL-6, suppressed t-PA expression.
*Conclusions:* We conclude that the proinflammatory cytokine IL-1β impairs fibrinolytic capacity in vascular endothelial cells by an NF-κB dependent suppression of t-PA expression. In contrast, an effect of IL-6 on t-PA expression could not be detected, probably due to lack of IL-6 receptor expression on HUVEC.
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Proinflammatory cytokines are known to have a number of effects which collectively render the circulation more susceptible to thrombosis (reviewed in [1]): Inflammatory cytokines may stimulate coagulation by causing a rise in circulating fibrinogen levels, an increased expression of tissue factor on endothelial cells and leukocytes as well as an induction of procoagulant phospholipid membrane surfaces. Moreover, inflammation suppresses the anticoagulant thrombomodulin/protein C system on endothelial cells. Inflammatory cytokines have also been reported to suppress fibrinolysis, mainly by induction of plasminogen activator inhibitor 1 (PAI-1) expression [2–5]. However, *in vivo* studies from our group indicate that the major determinant of the *local* fibrinolytic capacity is not the circulating levels of PAI-1 but the ability of the endothelium to release the key fibrinolytic enzyme tissue-type plasminogen activator (t-PA) [6]. Recent data from the Framingham Heart Study support the clinical importance of this mechanism, as T-allele carriers of the t-PA enhancer −7,351C/T polymorphism (who have a low capacity for t-PA release) have a more than 3-fold increased adjusted risk for myocardial infarction [7].

Despite its putative pathophysiological importance, relatively little is known about the specific effects of proinflammatory cytokines on t-PA expression in endothelial cells. In a recent study we showed that tumor necrosis factor alpha (TNF-α) suppresses t-PA mRNA expression in human vascular endothelial cells, mediated by nuclear factor (NF)-κB and p38 mitogen activated protein kinase (MAPK) signaling [8]. Regarding the inflammatory cytokine interleukin (IL)-1, the majority of studies report modest effects on t-PA antigen [2,3,9–11], whereas two studies show a decrease of secreted and cell-associated t-PA antigen following treatment of endothelial cells with IL-1 [4,5]. Data on the effects of IL-6 on t-PA expression in endothelial cells is limited to one study reporting lack of IL-6 effects on t-PA antigen levels [12].

In the present work we studied the effects of the proinflammatory cytokines IL-1β and IL-6 on t-PA expression in endothelial cells and investigated by which signaling mechanisms the effects on t-PA are mediated.

## Materials and methods

### Reagents

#### Cell culture

EGM-2 complete culture medium was from Clonetics/Cambrex (Walkersville, MD, USA), human recombinant IL-1β, IL-6 and soluble IL-6 receptor (sIL-6R) from R&D systems (Minneapolis, MN, USA), anti-human IL-6 polyclonal antibody from Pierce Biotechnology (Rockford, IL, USA), parthenolide from Sigma-Aldrich (St Louis, MO, USA), SP600125 from Calbiochem (Darmstadt, Germany), and SB203580 and PD98059 were obtained from Biosource (Nivelles, Belgium).

#### RT-PCR

E.Z.N.A Total RNA kit and RNase-free DNase I set were purchased from Omega Bio-tek (Doraville, GA, USA), and cDNA and Taqman reagents from Applied Biosystems (Foster City, CA, USA).

#### Enzyme-linked immunosorbent assay (ELISA)

t-PA antigen concentrations were analyzed using TintElize® t-PA obtained from Biopool International (Umeå, Sweden).

#### Western blot

Pre-cast 10% Tris-Glycine gels were from Cambrex (Rockland, ME, USA), antibody pairs for phosphorylated/total signaling proteins as well as the secondary horseradish peroxidase linked anti-rabbit IgG (#7074) were from Cell Signaling Technology (Danvars, MA, USA) with the following product numbers: anti-phospho-p65 #3033, anti-p65 #3034, anti-phospho-p38 MAPK #9215, anti-p38 MAPK #9212, anti-phospho-JNK #9251, anti-JNK #9252, anti-phospho-p44/p42 #9101 and anti-p44/p42 #9102. SuperSignal chemiluminescent substrate was from Pierce Biotechnology.

#### Electrophoretic mobility shift assay (EMSA)

Antibodies for EMSA supershifts of the NF-κB p50 (#sc-1190), p65 (#sc-7151) and c-Rel (#sc-70) were purchased from Santa Cruz Biotech (Santa Cruz, CA, USA).

#### Flow cytometry

Antibodies for flow cytometric analysis of IL-6Rα (CD126, # 551850) and gp130 (CD130, #552403) as well as streptavidin-APC (# 554067) were purchased from BD Biosciences (Erembodegem, Belgium).

### Cell culture and experimental design

Human umbilical vein endothelial cells (HUVECs) were prepared by collagenase treatment [13] of fresh umbilical cords obtained from the maternity ward of the hospital. The procedure of isolating HUVEC from umbilical veins was validated by FACS analysis for endothelial markers CD31 and CD34. Primary cultures were visually

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