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REGULATION OF PLASMINOGEN ACTIVATOR INHIBITOR-1 mRNA IN HUMAN ENDOTHELIAL CELLS. E.A. van den Berg (1), E. Sprengers (1), M. Jaye (2), W. Burgess (3), and V.W.M. van Hinsbergh (1). Gaubius Institute TNO, Leiden, The Netherlands (1), Meloy Laboratories, Springfield, VI, U.S.A. (2), and Biological Research Centre, Rockville, MD, U.S.A. (3).

Cultured human endothelial cells (HEC) increase their production of plasminogen activator inhibitor (PAI-1) upon stimulation with endotoxin and IL-1, agents that are known to cause an increase in PAI-1 levels in vivo. In order to study the regulation of PAI-1 synthesis at the mRNA level, we isolated a cDNA clone for the human PAI-1 gene from an endothelial expression cDNA library in λ gt 11 by screening with a PAI-1 specific antibody. Three positive cross-hybridizing clones were isolated. The longest insert (1500 bp) was partially sequenced (1000 bp). The sequence was identical to the PAI-1 sequence recently reported by others. The identity of the cDNA clone was further confirmed by comparison with part of the amino acid sequence of PAI-1. For that purpose t-PA-PAI-1 complex was purified from HEC conditioned medium by immunoadsorption to anti-t-PA IgG, and a suitable peptide was sequenced after comparison of the HPLC elution profiles of CNBr digests of t-PA and t-PA-PAI-1 complex. The amino acid sequence (M)FRQFQADFT completely matches the sequence predicted from the cDNA sequence.
By hybridization of the cDNA probe to Northern blots of total cellular RNA from human umbilical vein and artery EC (HUVEC, HUAEC), two transcripts of 2.3 and 3 kb were found. Primary HUAEC, incubated for 18 hours in growth medium, produced considerable although variable levels of PAI-1 activity and contained PAI-1 mRNA levels comparable to those found in subcultured HUAEC. When subcultured HUEC were incubated for 6 h with endotoxin, IL-1 or TNF, a 2-fold increase in PAI-1 mRNA was found with each of these mediators. Stimulation of the cells in the presence of cycloheximide resulted in a further increase of the 3 kb PAI-1 transcript. The 3' end of this transcript contains a 75 bp AT-rich sequence. Similar 3' AT-rich sequences have been found in mRNA's for a number of inflammatory mediators and cellular oncogenes, and in some cases it has been shown that removal of the sequence increased mRNA stability. The influence of cycloheximid on the larger PAI-1 transcript might be explained by inhibition of synthesis of a specific nuclease that controls the level of mRNA's harbouring such an AT rich sequence.

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TRANSIENT INDUCTION OF PLASMINOGEN ACTIVATOR INHIBITOR 2 (PAI-2) GENE TRANSCRIPTION BY THE TUMOUR PROMOTING PHORBOL ESTER PMA IN THE HUMAN MACROPHAGE-LIKE CELL LINE U-937. R.L. Medcalf, E.K.O. Kruithof and W.-D. Schleuning. Laboratoire central d'hématologie, CHUV, 1011 Lausanne, Switzerland.

The human hematopoietic cell line U-937 differentiates to a macrophage/monocyte phenotype after exposure to the tumour promoter PMA. We have recently shown that PMA concomitantly induces PAI-2 biosynthesis in these cells. Now, we have employed an 1880 base pair cDNA to study PAI-2 biosynthesis on the level of transcription and mRNA stability. By in vitro elongation of initiated PAI-2 transcripts in isolated nuclei in the presence of ^{32}p-labelled UTP followed by hybridization to cloned DNA ("run-on" transcription assay), we have demonstrated that PAI-2 gene transcription rates are induced 50-fold after exposure of the cells to PMA for 4 h in the presence of 5% fetal bovine serum. Transcription rates subsequently declined to base-line levels within 48 h. This transient increase of PAI-2 transcription rate was reflected by a transient induction of PAI-2 mRNA with maximal levels (60-fold) occuring after 16 h and subsiding to near base-levels after 48 h. Similar experiments performed in the absence of fetal bovine serum revealed that maximal levels of PAI-2 mRNA occured after only 4 hours exposure to PMA and were virtually absent after 16 h. Our results indicate that PMA induction of PAI-2 biosynthesis occurs at the level of gene transcription and is influenced by the presence of serum. Since PAI-2 is a major protein of U-937 cells, these date also suggest a role for hormonally modulated PAI-2 biosynthesis in macrophage physiology.

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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. V.W.M. van Hinsbergh (1), T. Kooistra (1), W. Fiers (2) and J.J. Emeis (1). Gaubius Institute TNO, Leiden, the Netherlands (1) and Laboratory of Molecular Biology, The State University of Gent, Belgium (2).

The vascular endothelium plays an important role in fibrinolysis by producing tissue-type plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI). The production of these factors by cultured endothelial cells (EC) is under separate control and influenced by various mediators, such as interleukin-1 (IL-1). Similar to IL-1, the monokine tumor necrosis factor (TNF) induces in EC various membrane bound components: tissue factor, HLA-A,B antigens and leukocyte adhesion molecules. We here report that TNF increased the production of PAI by human EC and increased PAI plasma levels in rats.
In the presence of serum, TNF increased the production of PAI by cultured human EC from umbilical vein (2-fold) and from foreskin microvessels (2 to 10-fold). This was demonstrated by titration of t-PA to a fixed amount of EC conditioned medium, by reverse fibrin autography, and by immunoprecipitation with specific anti-PAI-1 IgG. No change in t-PA activity was found by fibrin autography. The stimulation of PAI activity by TNF was found at 4 U/ml and reached a maximum at 500 U/ml; it was not prevented by the addition of polymycin B. Stimulation of PAI production by TNF or IL-1 was observed after 2 h and sustained for at least 24 h. Separate addition of TNF or IL-1 gave similar maximal stimulation of PAI production by EC at 500 U/ml and 5 U/ml, respectively, while the addition of both mediators resulted in a 2-fold larger increase. This indicates an additive effect of TNF and IL-1. TNF did not change PAI production by human hepatocytes.
To evaluate the effect of TNF in vivo, rats received a bolus injection of 250,000 U TNF/kg. Two h after injection, a 5-fold rise of circulating PAI levels was found (compared to control rats). Thereafter, the levels returned to basal values over a 10 h period. A decrease in circulating white blood cells was observed during the initial 3 h. The number of circulating platelets did not change.
We conclude that stimulation of the vascular bed by TNF not only results in a change in surface characteristics of the endothelium, but also can result in systemic changes. The increase in PAI levels by TNF may decrease fibrinolysis.

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EVIDENCE FOR A PLASMINOGEN ACTIVATOR INHIBITOR BINDING PROTEIN IN PLASMA B. Wiman, T. Carlsson and J. Chmielewska. Department of Clinical Chemistry, Karolinska Hospital, S-104 01 Stockholm, Sweden.

For several years it has been known that plasminogen activator inhibitor in plasma behaves as a high molecular weight compound on gelfiltration, in spite of that the molecular weight is only 50,000 in the presence of sodium dodecylsulphate. The reason for this has so far been unknown. On gel-filtration of plasma, to which purified latent PAI from HT 1080 cells was added, the PAI antigen gel-filtered as a 50,000 M_r protein. However, if the latent form of PAI was reactivated by guanidinium chloride prior to the gel-filtration experiment, an apparent molecular weight of about 250,000 for PAI antigen and activity was observed. If more than 10,000 U of PAI activity was added/mL of normal human plasma, excess PAI occurred at 50,000 M_r on gel-filtration. Human normalplasma was subjected to gel-filtration on sephacryl S-300 or Sepharose 6B and the fractions were checked for capacity to transform low M_r functional PAI to high M_r functional PAI. This capacity was only found in the 150 - 200,000 M_r region of the chromatogram. These data suggest that human plasma contains a protein that binds active forms of PAI. The complex of this protein and PAI could be dissociated by gel-filtration in the presence of 3 mol/L guanidinium chloride or 0.1% (w/v) sodium dodecylsulphate. The physiological or pathophysiological role of the PAI-binding protein is not known. Work with purification of the protein is in progress. Considerable purification have so far been obtained by precipitation with polyethylenglycol 6000 (0-6%), gel-filtration on Sephacryl S-300, followed by affinity chromatography on heparin-Sepharose.