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DIFFERENTIAL EXPRESSION AND SUBCELLULAR LOCALIZATION OF TISSUE FACTOR IN A CONSTITUTIVE VERSUS AN INDUCIBLE CELL TYPE. J.H. Morrissey, S.A. Gregory and T.S. Edgington. Research Institute of Scripps Clinic, La Jolla, CA, USA.

Tissue factor (TF) is an integral membrane glycoprotein and receptor present on a variety of cells outside of the vasculature, but normally absent from intravascular cells. TF plays a central role in initiation of coagulation by rapidly binding and allosterically activating bound factor VII/VIIa, which proteolytically activates coagulation factors IX and X. This protease cascade appears to play a role in the cellular inflammatory response, during which endothelial cells and monocytes/macrophages can be induced to express cell surface TF. Monocyte TF can be induced in response to endotoxin and also via direct interaction with activated T cells and by a specific lymphokine.

We have developed a panel of polyclonal and twenty-nine high affinity monoclonal antibodies to human TF. The antibodies recognize TF epitopes under a broad range of conditions, some of which rapidly and efficiently neutralize >95% of TF activity isolated from brain, placenta and expressed by cultured cells. Using these antibodies in immunohistochemical assays, we have observed little or no TF antigen cytologically associated with resting monocytes, no TF activity, and following stimulation, the cytologic appearance of TF antigen parallels the acquisition of TF activity. Immunohistochemical staining of stimulated monocytes is diffuse, consistent with homogeneous cell-surface distribution of the TF molecule. In addition, the normal human fibroblastic cell lines GM1380 and GM1381, which express TF constitutively, show a cytologically different and much more intense pattern of intracellular inclusions of TF. This is consistent with previous observations that lysed cells show about five-fold more TF activity than do intact cells. These findings indicate the presence of an intracellular storage site for TF in some cell types, a pattern presently associated only with constitutive expression of this receptor protein. In addition, they confirm that TF is induced in stimulated monocytes rather than translocation or modification. Supported by NIH grants HL-16411 and CA-41085.

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MOLECULAR CLONING OF HUMAN TISSUE FACTOR cDNA. T.S. Edgington, J.H. Morrissey and H. Fakhrai. Research Institute of Scripps Clinic, La Jolla, CA, U.S.A.

Tissue factor (TF), the cell-surface receptor and allosteric activator for factor VII/VIIa, is important in hemostasis and inflammation. The TF apoprotein was purified from human brain using factor VII-affinity chromatography and SDS gel electrophoresis, and was found to consist of a 47 kDa heavy chain plus a 12.5 kDa light chain. Approximately one-third of the heavy chain amino acid sequence was determined for four regions by microsequencing the intact protein and peptides derived from V8-protease digestion. A λgt11 cDNA library, made from mRNA derived from the human fibroblastic cell line WI38, was screened with (a) affinity-purified rabbit antibodies to human tissue factor, and (b) a 45-mer oligonucleotide probe based on TF heavy chain amino acid sequence. Five overlapping cDNA clones were identified and sequenced which confirmed all four partial TF amino acid sequences. Together these clones span the entire heavy chain coding sequence as well as 5' and 3' nontranslated regions. The N-terminus of the TF heavy chain is preceded by an unusually long signal peptide which appears to be cleaved at alternative sites two amino acids apart. This results in two variants of TF heavy chains which differ slightly in length and amino-terminal sequence. The deduced protein sequence shows no major homology to known protein sequences. However, a relatively uncommon tripeptide sequence, Trp-Lys-Ser (WKS), appears three times in the TF heavy chain. This tripeptide sequence also occurs in HMW kininogen, factor VIII, von Willebrand's factor and antithrombin-III. Limited sequence similarity is observed in flanking sequences, and this may indicate a possible functional domain for the recognition of members of the vitamin K-dependent serine protease family. Supported by NIH grants HL-16411 and CA-41085.



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THROMBOLYSIS BY TISSUE-PLASMINOGEN ACTIVATOR AND A FIBRIN(OGEN)-DEGRADATION PRODUCT, PEPTIDE 6A, IN A CANINE MODEL OF ELECTRICALLY-INDUCED CORONARY THROMBOSIS. T. Saldeen (1), J. Mehta (2), W. Nichols (2) and D. Lew (2). Department of Forensic Medicine, University of Uppsala (1) and Department of Medicine, University of Florida, USA (2).

Intracoronary thrombus resulting in acute myocardial ischemia can be lysed by thrombolytic agents, such as, streptokinase or t-PA. We examined the potential of a recombitant tissue-plasminogen activator (rt-PA) and a fibrin(ogen)-degradation product pentapeptide 6A, Ala-Arg-Pro-Ala-Lys, corresponding to aminoacids 43-47 in the Bß-chain of fibrinogen, which causes marked increase in coronary blood flow and stimulates prostacyclin release, in restoring coronary blood flow in dogs with experimentally-induced thrombus. An occlusive thrombus was created in the circumflex (Cx) coronary artery in 8 dogs by electrical stimulation of the endothelial surface. The electrically-induced Cx thrombus consisted primarily of platelets and fibrin. After the occlusive thrombus was stable without electrical current, rt-PA (10 ug/kg/minute for 30 minutes intravenously) or peptide 6A ( 5 umoles/minute for 20 minutes intracoronary) were randomly administered. Infusion of t-PA restored coronary blood flow (peak 22 ± 12 ml/minute, mean ± SD) in five of seven animlas. The time to flow restoration was 12.3 ± 9.1 minutes and the reflow persisted for 20.0 ± 10.9 minutes. Peptide 6A administration also restored coronary blood flow (peak 20 ± 4 ml/minute) in seven of eight animals with occlusive coronary thrombus. Mean time to blood flow restoration (4.3 ± 2.9 minutes) was shorter ($P<0.05$) than with rt-PA, but the reflow persisted only for the duration of the infusion (16.3 ± 10.2 minutes). Peptide 6A administration was associated with a significant ($P<0.05$) increase in plasma 6-keto-$PGF_1\alpha$ indicating stimulation of prostacyclin release. In addition, plasma t-PA concentrations also increased ($P<0.01$) at the peak effect of peptide 6A indicating release of endogenous t-PA as another potential mechanism of the thrombolytic effects of peptide 6A. This study demonstrates that peptide 6A exerts coronary thrombolytic effects compa rable to those of t-PA in a canine model of coronary thrombosis.

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EFFECT OF TISSUE-TYPE PLASMINOGEN ACTIVATOR (t-PA) ON BACTERIAL ENDOCARDITIS. A.G.M. Buiting (1), J. Thompson (1), J.J. Emeis (2), H. Mattie (1), E.J.P. Brommer (2), R. van Furth (1). Infectious Diseases Department, University Hospital (1), and Gaubius Institute TNO (2), Leiden, The Netherlands.

In bacterial endocarditis the causing microorganisms are located in a fibrin-platelet matrix, making them less accessible to host-defence mechanisms and antibiotic therapy. Trombolytic treatment could break down the fibrin of these endocardial vegetations and thus eliminate the focus of infection. This approach was studied in vitro and in vivo using recombinant t-PA (rt-PA, Wellcome Biotech) having a fibrinolytic activity comparable with melanoma t-PA. The following results were obtained.

1. Incubation of Streptococcus sanguis infected plasma clots in the presence of t-PA resulted in lysis of the clots as evidenced by a significant decrease in the weight of the clots and by an increase in the number of streptococci in the medium. No effect of t-PA was found on the antimicrobial action of penicillin G (PenG) on the streptococci in the non-lysed part of the clots.
2. Vegetations isolated from the heart of rabbits with a S. sanguis endocarditis, incubated in medium with t-PA were also lysed. This resulted in a rise in the medium of both the number of bacteria and the concentration of fibrin degradation products.
3. Treatment of rabbits with a S. sanguis endocarditis using a combination of rt-PA, given as bolus injections at one hour intervals (4x0.5 or 4x1 mg per kg/day), and PenG decreased the weight of the endocardial vegetations significantly compared to a control group treated with PenG only (43.3 and 81.8 mg respectively). No additional decrease in weight was obtained with repeated administration of t-PA at daily intervals up to 2 days. The significant decrease of the number of streptococci per gram of vegetation as a result of the treatment with PenG was not influenced by t-PA. Treatment with 4x1 mg t-PA per kg caused a small decrease (about 30%) in the plasma concentration of plasminogen, fibrinogen and $\alpha_2$-antiplasmin. However, no bleeding complications were observed.

In conclusion rt-PA can influence the treatment of bacterial endocarditis in rabbits by decreasing the size of the vegetations but not by influencing antimicrobial action on the bacteria in the non-lysed part of the vegetation.