7. Summary

In the pathogenesis of vascular disease, inflammation and coagulation play a pivotal role. Inflammatory processes have been shown to alter the hemostatic balance of endothelial cells (ECs). Various studies demonstrated proinflammatory cytokines to induce tissue factor (TF), known as the initiator of the extrinsic coagulation pathway in ECs, thereby increasing their thrombogenic potential. Recently, alternatively spliced TF (asTF), a soluble isoform of TF, has been discovered, possibly contributing to procoagulability. Whether ECs express this TF variant and whether there are agonists that induce asTF expression and its release, is yet unknown. Therefore, this study examined the effects of the proinflammatory cytokines tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and on the endothelial expression of this TF variant and delineates the impact of asTF on the procoagulability of extracellular fluids in comparison with the procoagulant potential of microparticle (MP)-associated TF.

AsTF mRNA was found to be rapidly induced after stimulation with proinflammatory cytokines, whereas induction of full-length TF mRNA expression was delayed, pointing to asTF to be differentially expressed after cytokine treatment. AsTF was released from endothelial cells and exhibited procoagulant activity in the presence of phospholipids. Removal of asTF from the supernatant revealed the procoagulant activity to be asTF associated.

Results obtained from experiments using specific inhibitors suggested alternative splicing of TF to be mediated via phosphorylation of SR-proteins by CDC2-like kinase (CLK).

Application of ionizing radiation (IR) is noted to be associated with organ thrombosis and fibrosis which are of clinical importance. However, IR-induced TF expression has not been linked to the occurrence of late thrombosis yet. In a second step the expression of both TF isoforms was thus examined in response to IR. A persistent induction of TF and asTF after IR was observed. Combined stimulation with IR and TNF-α led to an immense shedding of MP-associated TF. Comparing the procoagulability of asTF and TF-bearing MP, the soluble TF isoform, despite its ability to exhibit procoagulant properties in a phospholipid-rich environment, seems only to be of lesser importance for the the cellular procoagulability. With regard to its early release from ECs in response to TNF-α asTF may become important as a prognostic marker for an imbalanced endothelial hemostasis under inflammatory conditions.

In addition, this study further investigated the effects of the antioxidants pyrrolidine dithiocarbamate (PDTC) and N-acetylcyseine (NAC) on the IR- and TNF-α induced expression of TF and its release from ECs. Antioxidative pretreatment markedly reduced ROS formation and inhibited apoptosis, thereby significantly reducing the shedding of thrombogenic microparticles. Thus, antioxidants may help to prevent thrombotic events after antiproliferative treatment such as IR or under inflammatory conditions such as sepsis respectively when used in combination with anticoagulants.

These results may be a base for further investigations regarding the (patho-) physiological relevance of the alternatively spliced TF and its suitability as a clinical marker.