Prothrombotic effects of tumor necrosis factor alpha in vivo are amplified by the absence of TNF-alpha receptor subtype 1 and require TNF-alpha receptor subtype 2.

Elevated serum levels of the proinflammatory cytokine tumor necrosis factor alpha (TNF?) correlate with an increased risk for atherothrombotic events and TNF? is known to induce prothrombotic molecules in endothelial cells. Based on the preexisting evidence for the impact of TNF? in the pathogenesis of autoimmune disorders and their known association with an acquired hypercoagulability, we investigated the effects of TNF? and the role of the TNF receptor subtypes TNFR1 and TNFR2 for arteriolar thrombosis in vivo.

Arteriolar thrombosis and platelet-rolling in vivo were investigated in wildtype, TNFR1-/-, TNFR2-/- and TNFR1-/R2-/- C57BL/6 mice using intravital microscopy in the dorsal skinfold chamber microcirculation model. In vitro, expression of prothrombotic molecules was assessed in human endothelial cells by real-time PCR and flow cytometry. In wildtype mice, stimulation with TNF? significantly accelerated thrombotic vessel occlusion in vivo upon ferric chloride injury. Arteriolar thrombosis was much more pronounced in TNFR1-/- animals, where TNF? additionally led to increased platelet-endothelium-interaction. TNF? dependent prothrombotic effects were not observed in TNFR2-/- and TNFR1-/R2- mice. In vitro, stimulation
of human platelet rich plasma with TNF? did not influence aggregation properties. In human endothelial cells, TNF? induced superoxide production, p-selectin, tissue factor and PAI-1, and suppressed thrombomodulin, resulting in an accelerated endothelial dependent blood clotting in vitro. Additionally, TNF? caused the release of soluble mediators by endothelial cells which induced prothrombotic and suppressed anticoagulant genes comparable to direct TNF? effects. TNF? accelerates thrombus formation in an in vivo model of arteriolar thrombosis. Its prothrombotic effects in vivo require TNFR2 and are partly compensated by TNFR1. In vitro studies indicate endothelial mechanisms to be responsible for prothrombotic TNF? effects. Our results support a more selective therapeutic approach in anticytokine therapy favouring TNFR2 specific antagonists.