Role of toll-like receptor 4 on tissue factor regulation in human monocytes

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Funding Acknowledgement: Type of funding source: None

Background: Inflammation and coagulation play a pivotal role in the pathogenesis of acute coronary events and an extensive cross-talk links the two systems, whereby inflammation activates coagulation and coagulation affects inflammatory activity. Infact, pro-inflammatory stimuli can induce tissue factor (TF) expression, the principal initiator of the clotting cascade, in circulating monocytes and activate pathways leading to thrombin generation.In turn, TF may bind cellular receptors which may affect the production and release of inflammatory mediators. According to our recent results, proprotein convertase subtilisin/kexin9 (PCSK9) and Gamma-Glutamyltransferase (GGT), two molecules involved in the pathogenesis of cardiovascular disease, are able to up-regulate TF expression in monocytes by activating NFkB pathway but the mechanism and the receptor involved in this biological response is unknown. One plausible possibility is that both molecules bind a Toll-like Receptor (TLR)4 located on membranes of human and cultured monocytes,activating the TLR4/MyD88-NFkB pathway and eventually leading to stimulation of TF expression.

Aim: To assess whether both molecules are able to bind to TLR4 located on the surface of human monocytes and whether this specific binding involves the TLR4/MyD88-NFkB pathway on TF modulation.

Methods: THP-1 cells, a human monocytic cell line derived from an acute monocytic leukemia patient, was used as in vitro model. The cells were stimulated with human (h) recombinant (r) PCSK9 (5 μg/ml) and hrGGT (1 μg/ml) or pre-incubated with BAY-117082 (BAY, 10–5M) a NFκB inhibitor, CLI-095 ($3x10^{-6}$M), a highly Myd88/TLR4 signaling specific inhibitor and LPS-RS (1 μg/ml) a TLR4 antagonist. TF procoagulant activity (PCA), was assessed by 1-stage clotting assay and the results expressed by ρg/mL of active protein. Experimental series were carried out in endotoxin-free conditions, in order to exclude lipopolysaccharide (LPS)-dependent immune responses.

Results: hrPCSK9 and hrGGT stimulated TF expression (PCA: from 50±20 to 120±20, n=10, $p<0.01$) and (PCA: from 190±140 to 460±360, n=15, $p<0.001$) respectively, an effect down-regulated by BAY,a NFκB inhibitor (PCA by hrPCSK9: –71±23%, n=5, $p<0.01$; PCA by hrGGT: –90±21%, n=7, $p<0.001$). CLI-095, a TLR4 inhibitor (PCA by hrPCSK9: –86±26%, n=3, $p<0.05$; and PCA by GGT: –89±10%, n=5, $p<0.001$).LPS-RS, a TLR4 antagonist, (PCA by hrPCSK9: –74±25%, n=3, $p<0.05$; PCA by hrGGT: –70±17%, n=5, $p<0.001$) abolished both PCSK9 and GGT-induced TF expression.

Conclusions: These data are the first demonstration of a direct role of PCSK9 and GGT as active mediators of inflammatory-based thrombotic diseases. The possible mechanism of action involves recognition of two proteins by TLR4 on monocytes membrane surface, lead to activation of the transcription factor NFκB. Further studies will be needed to better understand the regulatory mechanisms underlying this complex set of biological responses that bind TLR4 modulation and TF expression.