Binding of gamma-glutamyl transferase to TLR-4 allows the activation of tissue factor expression in human monocytes

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Background: Gamma-glutamyl transferase (GGT) plays a key role in the antioxidant processes, however, it also exerts pro-oxidant effects by activating NFkB, a redox-sensitive transcription factor key in the induction of Tissue Factor (TF) gene expression, the initiator of the clotting cascade. GGT may modulate TF expression, an assumption verified by previous studies carried out in human Peripheral Blood Mononuclear Cells (PBMCs). Quite importantly, TF expression in response to GGT stimulation was independent of its enzymatic activity since those experiments were conducted by using human recombinant (hr)GGT, a wheat germ-derived protein enzymatically inert. Thus, GGT may act through a cytokine-like mechanism although the precise determinants of its action and the receptor involved were not defined by those experiments.

Purpose: To assess whether GGT-induced TF stimulation is a consequence of binding to Toll-Like Receptor (TLR)-4 and activation of NF-κB, as suggested by results recently obtained in different experimental contexts.

Methods: PBMCs obtained from healthy donors through a discontinuous Ficoll/Hystopaque density gradient and THP-1 cells, a human monocytic cell line derived from a leukemia patient, were incubated with hrGGT (0.5 ng/µl for PBMCs and 1ng/µl for THP-1). LPS-Rs (0.5 ng/µl for PBMCs and 1 ng/µl for THP-1), CLI-095 ($3x10^{-6}$ M) and BAY-11-7082 (10^{-5} M) were used to block TLR-4 receptors, TLR4 signaling and NF-κB respectively. TF pro-coagulant activity (PCA) was assessed using of StartMax coagulometer and results were expressed in pg/ml after calibration with a standard curve. HEK-Blue hTLR4-positive and HEK-Blue hTLR4-negative cells are used to evaluate the engagement of TLR4 by hrGGT.

Results: hrGGT increased TF expression in both PBMCs (PCA from 110±70 to 510±43, n=7, $p<0.01$) and THP-1 cells (PCA from 170±64 to 460±80, n=15, $p<0.001$).In PBMCs GGT-induced TF stimulation was antagonized by LPS-Rs (PCA: –72±17% n=4, $p<0.01$) a TLR-4 antagonist, CLI-095 (PCA:-74±34%, n=7, $p<0.001$) a TLR-4 intracellular antagonist and BAY-11-7082 (PCA: –71±32%, n=7, $p<0.001$), a NF-κB inhibitor. Similar results were obtained in THP-1 cells [LPS-Rs: –76±15%, n=6, $p<0.01$; CLI-095: –100±6,6%, n=6, $p<0.01$; BAY-11-7082: –100±2,1%, n=6, $p<0.01$]. hrGGT activates NF-κB in hTLR4-positive HEK cell lines while doesn't induces effect in TLR4-negative HEK cells.

Conclusions: Besides confirming the cytokine-Like activity of GGT and its procoagulant effect in PBMCs and THP-1 cells, these data identify for the first time the possible role of TLR-4 as the receptor of GGT and NfkB as the involved signal transduction pathway. The GGT-TLR-4 link may provide an explanation to the association between circulating GGT levels and increased risk of acute thrombotic events as well as to the involvement of GGT in the morbid evolution of the atherosclerotic plaque in which GGT colocalizes with monocytes and foam cells, the prime sources of TF within the plaque.