Macrophages Activation by Heparanase: Possible Role in Vulnerable Plaque

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Background: Activated macrophages have a role in vulnerable plaque(VP), which results in ischemia and myocardial infarction. Factors and mechanism that activate macrophages in the plaque are incompletely understood. Heparanase (Hpa) is endoglycoside associated with angiogenesis and inflammation, which are characteristic of VP. The aim of this study is to examine possible involvement of Hpa in VP.

Methods: Highly purified Hpa protein and variants were added to mouse peritoneal macrophages (MPM) and macrophage- like cell line J774. Medium was collected after 24 hours and examined by ELISA for the level of TNF-alpha, MMP-9, IL-1, and MCP-1. Gene expression of the same proteins was determined by RT-PCR. Cells collected from Toll like receptor (TLR) 2-and 4 knockout mice were similary activated. Phosphorylation of Akt, Erk and I kappa B was evaluated by immunoblotting before and after incubation with Hpa.

Results: Incubation of J774, and MPM with latent 65 kD Hpa resulted in marked increase in TNF alpha (57±43 vs 3933±2047 pg/ml, $p< 0.001$), MMP-9 (5.3±3 vs 17.6±8.3 pg/ml, $p< 0.001$), IL-1 (0±0 vs 6.8± 2.2 pg/ml, $p= 0.005$) and MCP-1 (100± 11 vs 2336± 682 ng/ml, $p=0.004$) levels. Nearly the same effect was observed following the addition of enzymatically inactive Hpa (DM) or the Hpa C – terminus domain. Proteolytic digestion of these proteins abolished cytokine elevation. Cytokine secretion was attenuated by inhibitors of MAPK and PI3K pathways and by inhibitors of NF-kappa B. MPM harvested from TLR- 2 and 4- knockout mice were not activated by Hpa and neutralizing anti TLR- 2 and 4 antobodies reduced cytokines induction by Hpa.

Conclusion: Hpa activates macrophages, by increasing the expression and secretion of TNF alpha, MMP-9, IL-1 and MCP-1. This process is mediated by TLR 2 and 4, signaling via PI3K and MAP-K pathways and activation of NF kappa B, suggesting that heparanase play a role in inflammatory responses and plaque vulnerability.