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Innate immune modulation in atherosclerosis - with focus on ApoB100 derived danger associated signal 1 (ApoBDS-1)

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ABSTRACT

Elevated level of LDL is the most important risk factor for atherosclerosis. ApoB100 is the only unexchangeable protein in LDL particle. Recent reports have shown that native peptides of ApoB100 trigger activation of adaptive immune responses. Whether ApoB100 can activate innate immune response is less-known.

In this thesis, we identified a native ApoB100 peptide from human ApoB100, named ApoB100 danger associated signal-1 (ApoBDS-1), given its biological nature to trigger innate proinflammatory responses in monocytes and macrophages. Besides macrophages, ApoBDS-1 can also activate platelets and endothelial cells, eliciting proinflammatory mediators and promoting platelet-leukocyte aggregates through complex molecular mechanisms involving Ca^{2+} flux, ROS production, MAPKs activation, PI3K-Akt activation, and microRNA regulations. ApoBDS-1 contributes to the activation of inflammatory signaling in human atherosclerotic plaque. We showed that ApoBDS-1 exists in human carotid plaques by immunofluorescence staining. Size-exclusion chromatography and Western blot confirmed that some low molecular weight fractions isolated from plaque contain ApoBDS-1 epitopes and possess ApoBDS-1-like bioactivity for induction of IL-8. These findings suggest that active ApoBDS-1 presents in atherosclerotic lesions. Analysis of BiKE database indicates that inflammasome pathways are involved in atherosclerosis and associated with the disease severity. Our studies show that ApoBDS-1 is an endogenous activator of NLRP3 inflammasome, inducing IL-1β in monocytes and macrophages via NLRP3-dependent caspase-1 activation. We also found that ApoBDS-1 could induce NLRP3 inflammasome complex formation in vivo, and activate NLRP3 inflammasome by induction of K^+ efflux. Lastly, we explored the receptor/interacting protein for ApoBDS-1 using far Western blot and 2-D electrophoresis and identified TNF receptor associated protein 1 (Trap1) as an ApoBDS-1 specific interacting protein. Trap1 and ApoBDS-1 are colocalized mainly in cytoplasm and also on cell surface membrane. Biacore SPR analysis suggests that ApoBDS-1 binds to Trap1 with a medium affinity depending on the last 5 amino acids in its C-terminal domain. Trap1 is indispensable for ApoBDS-1 function since ApoBDS-1 induced cytokine secretion and reactive oxygen species can be inhibited by Geldanamycin, an inhibitor of Trap1 or by knocking down of Trap1 using specific shRNA.

Taken together, we have identified ApoBDS-1 as the innate immune activator in ApoB100. Blocking the interaction of ApoBDS-1 and Trap1, or inhibition of ApoBDS-1 induced signaling pathways may represent new therapeutic options for atherosclerosis treatment.