NLRP3 INFLAMMASOME-MEDIATED IL-1β PRODUCTION BY LPS-PRIMED GM-MFs IS INDEPENDENT OF P2X7 RECEPTOR

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Introduction: IL-1β is one of the main inflammatory cytokines that regulates immune responses. The main source of this cytokine is the activated macrophages. IL-1β is synthetised as a proprotein, which is proteolytically processed to its active form by an intracellular protein complex, called NLRP3 inflammasome. Activation of NLRP3 inflammasome requires two signals: the first signal leads to the synthesis of pro-IL-1β and the components of the inflammasome, the second signal, like the extracellular ATP, results in the assembly of the NLRP3 inflammasome and the cleavage of pro-IL-1β. Under metabolically stressful conditions like inflammation and cell damage ATP is released into the extracellular space via pannexin channels. The extracellular ATP is sensed by P2X7 receptor and the activation of the P2X7 leads to K^+ efflux.
Methods: Human monocytes were separeted from human peripheral blood and were cultured for five days in presence of GMCSF to become inflammatory macrophages (GM-MFs). The macrophages were activated by LPS. The cytokine production was measured by ELISA.
Results: Our results show that GM-MFs can secrete substantial amounts of mature IL-1β upon stimulation with LPS in the absence of ATP stimulation. Previous studies have shown that human monocytes can release mature IL-1β with LPS stimulation alone, which is dependent on autocrine stimulation by ATP. LPS-primed GM-MFs can also release ATP, but the addition of apyrase, an enzyme that hydrolyzes extracellular ATP, do not affect IL-1β secretion, which is consistent with the lack of requirement for P2X7 receptor or pannexin inhibitor as well as the high extracellular K^+ concentration.
Conclusion: To summarize our results we can determine that the accepted two-signal model necessary for activation of the NLRP3 inflammasome in response to TLR ligands does not apply to GM-MFs and other mechanisms may play role in the secretion of IL-1β by LPS primed GM-MFs in the absence of extracellular ATP.