Mitochondria involvement in NLRP3 inflammasome pathways in monocytes and endothelial cells

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NLRP3 inflammasome activation provides an innate host defence against pathogens either via interleukin-1β synthesis or by pyroptosis. Two steps of inflammasome complex activation are recognised: Signal 1 or priming (expression of NLRP3 protein, pro-IL-1b, pro-IL-18 and pro-Caspase-1), followed by Signal 2 or activation (active IL-1b and IL-18). Additionally, Caspase 5 may be involved via a non-canonical pathway. The purpose of this study was to investigate the possible role of mitochondrial and cytosolic superoxide in activating the NLRP3 inflammasome in THP-1 and HUVEC and EA.hy926 endothelial cells. THP-1 cells were differentiated with PMA (5ng/ml) for 1-3 days. Subsequently, LPS treatment (0.1µg/ml) was administered for 24 h for Signal 1 activation followed by ATP (500uM) for 1 hour for Signal 2. The effects of intracellular generation of superoxide (mitoparaquat and paraquat at 1 and 5µM) was investigated before and after LPS. NLRP3, pro-IL-1β, pro-IL-18, pro-Caspase-1 and -5 were detected in THP-1 cell lysates by Western blotting and active IL-1β and IL-18 detected in cell culture supernatants by ELISA. In our hands, intracellular superoxide generation did not activate the NLRP3 inflammasome in PMA-differentiated THP-1 cells nor did it inhibit LPS-induced priming. Both endothelial cell types showed evidence of low level Signal 1 activation. Despite HUVEC cells showing priming of the NLRP3 inflammasome, there was no evidence for either active IL-1β or active IL-18 synthesis. Surprisingly, EA.hy926 cells showed low levels of active IL-1β, when exposed to PMA, suggesting other endpoints of an active NLRP3 inflammasome are worthy of investigation.