

**TYROSINE PHOSPHATASE SHP-1 REGULATES LPS-INDUCED
CYTOKINE NETWORKS INVOLVED IN INFLAMMATION**

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The role of the SH2-domain containing protein tyrosine phosphatase, SHP-1 in the regulation of signal transduction pathways activated by cytokine, antigen and growth factor receptors has been studied for some time. However, the involvement of this tyrosine phosphatase in the regulation of LPS-activated signal transduction pathways leading to cytokine production is less understood. Motheaten (*me/me*) mice, homozygous for the recessive allelic mutations in the SHP-1 gene, develop severe immune dysfunction, associated with chronic inflammation and autoimmunity. The “Motheaten condition” is characterized by an expansion of myeloid cells and their infiltration into various tissues resulting in alopecia, splenomegaly, pneumonitis and premature death. Since macrophages are a predominant source of inflammatory cytokines and, the loss of SHP-1 results in their hyper-proliferation and hyper-activation, we hypothesized that chronic inflammation observed in *me/me* mice is caused by a deregulated production of pro-inflammatory cytokines and deregulated cytokine networks. Stimulation of monocytic cells with LPS induces first pro-inflammatory (IL-1, IL-6, TNF- α , etc.) and then anti-inflammatory (IL-10, sTNF-R and IL-1R antagonist) cytokines. To get insights into the involvement of SHP-1 in CD14/TLR4-mediated signal transduction pathways leading to cytokine production, we determined, by ELISA and RT-PCR, the levels of pro-inflammatory and immuno-regulatory cytokines in culture supernatants of normal and *me/me* derived splenic macrophages following stimulation with LPS. Our results show enhanced levels of TNF- α mRNA and the protein in LPS stimulated *me/me* splenic macrophages compared to normal controls. In contrast, the levels of IL-10, an immuno-regulatory cytokine, implicated in the inhibition of pro-inflammatory cytokine production, were significantly reduced. Both elevated levels of TNF- α and diminished IL-10 production were observed irrespective of LPS dose and duration of LPS stimulation. Addition of exogenous rIL-10 to *me/me* macrophages cultures could lower, in a dose dependent manner. To understand the events leading to down-regulation of IL-10 in LPS-stimulated SHP-1 deficient murine macrophages, we investigated the role of p38 and ERK MAP kinases by using their specific inhibitors. SB202190, the p38-specific inhibitor, inhibited LPS-induced IL-10 production in both normal and *me/me* macrophages. In contrast, PD98059, a specific inhibitor of ERK kinases, failed to modulate IL-10 production in normal macrophages but

partially inhibited IL-10 production in *me/me* splenic macrophages. In summary, our results reveal a critical role for the tyrosine phosphatase SHP-1 in CD14/TLR4 signal transduction pathways regulating cytokine networks and suggest that the elevated levels of TNF- α secretion by *me/me* macrophages may be a consequence of diminished IL-10 production by these cells. Our data support the role of p38 MAPK in IL-10 production, and further suggest that Erk1/2 MAPKs may also be partially involved in LPS induced signal transduction pathways regulating IL-10 production.