TLR4-mediated activation of pro-inflammatory cell signaling is attenuated in P2Y2-/- bone marrow-derived macrophages

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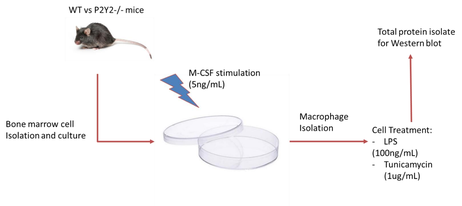
BACKGROUND

- Endotoxin (LPS)-mediated activation of Toll-like receptor 4 (TLR4) signaling in hepatic macrophages has been implicated in the pathogenesis of non-alcoholic steatohepatitis (NASH)
- TLR4-mediated induction of pro-inflammatory cytokine and chemokine synthesis is dependent on efficient induction of MAPK and NF-kB signaling and endoplasmic reticulum (ER) stress in macrophages
- Excessive fat accumulation within hepatocytes results in hepatocellular injury and elevated ATP levels in the extracellular milieu.
- Extracellular ATP-mediated activation of P2Y2 purinergic receptors has the potential to influence macrophage polarization and pro-inflammatory cytokine and chemokine synthesis and secretion.
- Previous studies in our laboratory suggests that TLR4-mediated induction of pro-inflammatory cytokine and chemokine expression is attenuated in P2Y2 -/- bone marrow-macrophages (Narayanan et al, BCM Pediatric Research Symposium, 2018)
- The purpose of this study is to gain further mechanistic insights into the role of P2Y2 purinergic signaling and its interaction with LPS/TLR4-mediated induction of pro-inflammatory mediators (ERK, JNK and p38 MAPK, NF-kB signaling and ER stress) in macrophages.

Hypothesis

P2Y2 purinergic receptor expression is essential for the efficient induction of LPS/TLR4-mediated activation of pro-inflammatory signaling in macrophages

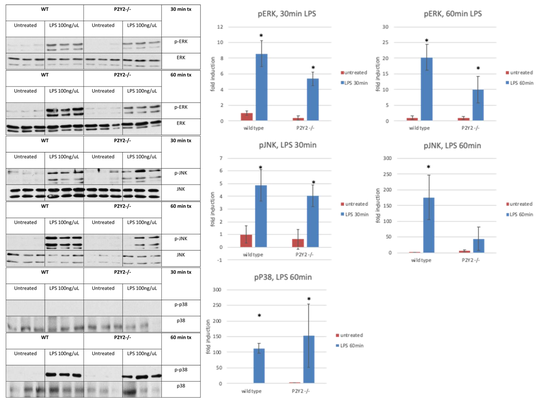
METHODS



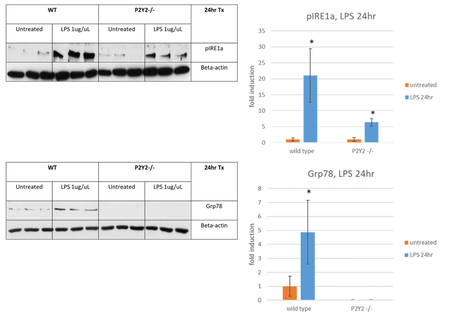
Equal protein loading was assessed by re-probing blots with antibody specific for β–actin. Fold activation was calculated based on the densitometric analysis of band intensities and statistical significance was determined by unpaired student t-test.

LPS/TLR4-mediated phosphorylation and activation of ERK and JNK MAPK signaling is attenuated in P2Y2 -/- BMDM.

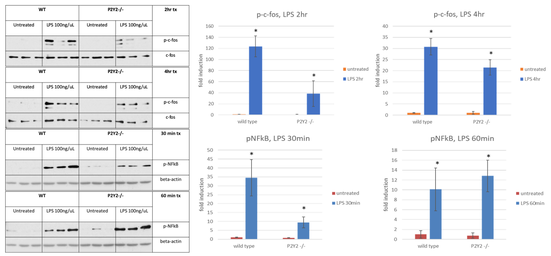
Activation of p38 MAPK is comparable between the WT and P2Y2-/- BMDM



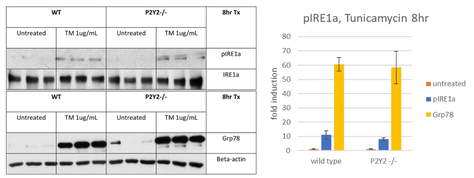
LPS/TLR4-mediated induction of ER Stress, (phosphorylation of IRE1α and induction of Grp78 is attenuated in P2Y2-/- BMDM



LPS/TLR4 mediated phosphorylation and activation of c-fos and NF-kB (key transcriptional regulators of proinflammatory cytokine synthesis) are attenuated in P2Y2 -/- BMDM



Tunicamycin-mediated induction of phospho-IRE1α and Grp78 are comparable between the WT and P2Y2-/- BMDM



RESULTS

- LPS/TLR4-mediated induction of phosphorylation and activation of ERK MAPK signaling was attenuated in P2Y2-/- BMDMs (FC, WT vs P2Y2 -/-, LPS [30 min]: 8.5, p<0.05 vs 5.3, p<0.05; LPS [60 min]: 20, p<0.05 vs 10, p=0.05)
- LPS/TLR4-mediated induction of phosphorylation and activation of JNK MAPK signaling were attenuated in P2Y2-/- BMDMs (FC, WT vs P2Y2 -/-, LPS [30 min]: 5.5, p<0.05 vs 5.6, p=0.2; LPS [60 min]: 135, p<0.05 vs 54, p=0.19)
- LPS/TLR4-mediated induction of phosphorylation and activation of p38 signaling was comparable between the genotypes (FC, WT vs P2Y2 -/-, LPS [60 min]:112, p<0.05 vs 153, p=0.12)
- LPS/TLR4-mediated induction of phosphorylation and activation of c-fos (a key target of MAPK signaling) was attenuated in P2Y2-/- BMDM (FC, WT vs P2Y2 -/-, LPS [2 hr] : 123, p<0.05 vs 38, p=0.1; LPS [4 hr]: 30, p<0.05 vs 21, p<0.05).
- LPS/TLR4-mediated phosphorylation and activation of NF-kB signaling was attenuated at 30 min in P2Y2-/- BMDM, as compared to WT, with comparable activation observed between genotypes at 60 min, suggesting a role for P2Y2 purinergic receptor function in the temporal regulation of NF-kB signaling in macrophages (FC, WT vs P2Y2 -/-, LPS [30 min]: 34.5, p<0.05 vs 9.4, p<0.05; LPS [60 min]: 10, p<0.05 vs 12, p<0.05)
- LPS/TLR4-mediated induction of ER stress, as assessed by the upregulation of IRE1α and Grp78 was attenuated in P2Y2-/- BMDMs (FC, WT vs P2Y2 -/-, IRE1α LPS [24 hr]: 21, p=0.05 vs 6.3, p<0.05); (FC, WT vs P2Y2 -/-, Grp78, LPS [24 hr]: 5.6, p=0.09 vs 0.03, p<0.05)
- Tunicamycin (a known inducer of ER stress)-mediated induction of IRE1α and Grp78 were comparable between the genotypes (FC, WT vs P2Y2 -/-, TM [8 hr], IRE1α: 11, p<0.05 vs 8, p<0.05); Grp78: 60, p=0.09 vs 58, p<0.05)

CONCLUSION

LPS/TLR4-mediated activation of MAPK (ERK, JNK) and NF-kB signaling and the induction of ER stress were dependent on intact P2Y2 purinergic signaling in bone marrow-derived macrophages.

Our results suggest a novel interaction between cellular damage sensors (P2Y2 purinergic receptors) and pathogen sensors (TLR4) in the co-ordinate regulation of macrophage activation.

In summary, P2Y2 purinergic signaling may play a role in the modulation of LPS/TLR4-emediated induction of the pro-inflammatory phenotype of macrophages with implications for the pathogenesis of NASH.

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