GLI3 mediates TLR-induced inflammatory response

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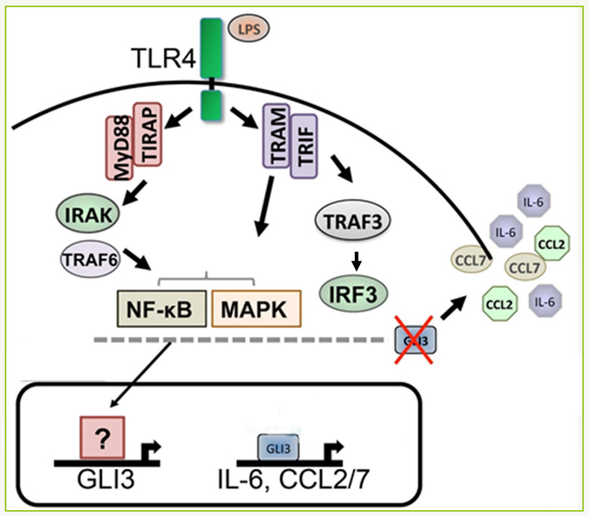
Abstract

While acute inflammation is important for a healthy immune response, chronic inflammation has emerged as a mechanism leading to formation of many diseases including autoimmune and malignant disorders. Monocytes are a subset of innate immune cells that secrete inflammatory cytokines in response to infections through toll-like receptors (TLRs). In this study, we found that stimulation of human monocyte cell lines and peripheral blood monocytes with the TLR4 ligand, lipopolysaccharide (LPS), induces an increase in mRNA and protein expression of the transcription factor GLI3.Pharmacological inhibition of TLR4 significantly reduces GLI3 expression. Using dominant negative forms of MyD88/TIRAP or TRIF/TRAM and RNAi for MYD88 which antagonize these signaling molecules, we found that TLR4-mediated regulation of GLI3 occurs through TRIF- but not MyD88- mediated signaling. Our data also suggest that ectopic GLI3 expression induces a similar set of inflammatory cytokines as TLR4-signaling suggesting GLI3 may mediate TLR4-induced inflammation. This includes CCL2, CCL7 and IL-6 among other inflammatory cytokines. Further analysis of the molecular mechanism of GLI3 regulation of inflammatory cytokines will extend our understanding of the interaction between TLR4 signaling and GLI transcription factors.

Background



Results

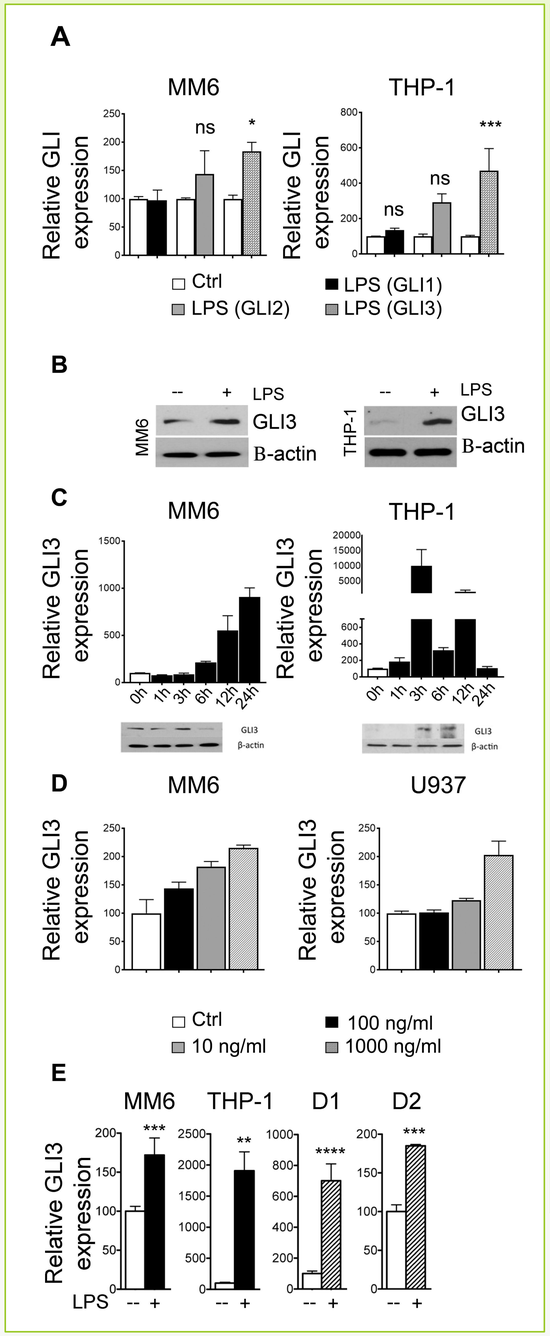


Figure 1: A) qPCR analysis of LPS treated monocytes suggest an increase in GLI3 mRNA expression **B)** WB analysis of LPS treated cells suggests an increase in GLI3 protein expression **C)** qPCR and WB analysis of GLI3 mRNA and protein expression in a time dependent manner. **D)** qPCR showing a dose dependent increase in GLI3 expression following LPS challenge **E)** Elevated GLI3 expression in primary monocytes from peripheral blood of healthy donors treated with LPS.

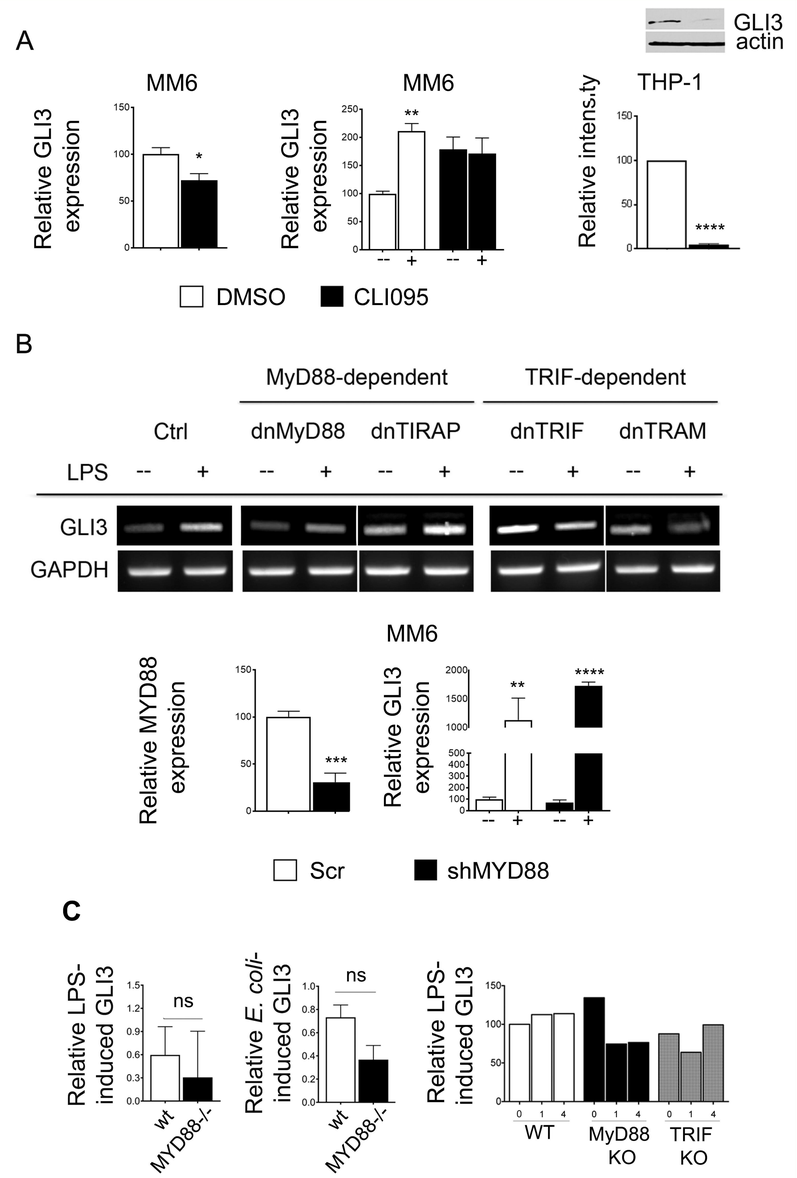


Figure 2: A) mRNA and WB analysis of monocytes treated with the TLR4 inhibitor (CLI095) **B)** RT-PCR and qPCR analysis of LPS treated MM6 cells transfected with dominant negative (dn) forms of TLR4 signaling or RNAi targeting of MYD88. **C)** Relative induction of GLI3 expression in wt and MYD88 KO macrophages extracted from **GSE1405**. Relative induction of GLI3 expression in wt,MYD88 KO and TRIF KO macrophages extracted from **GSE14890**

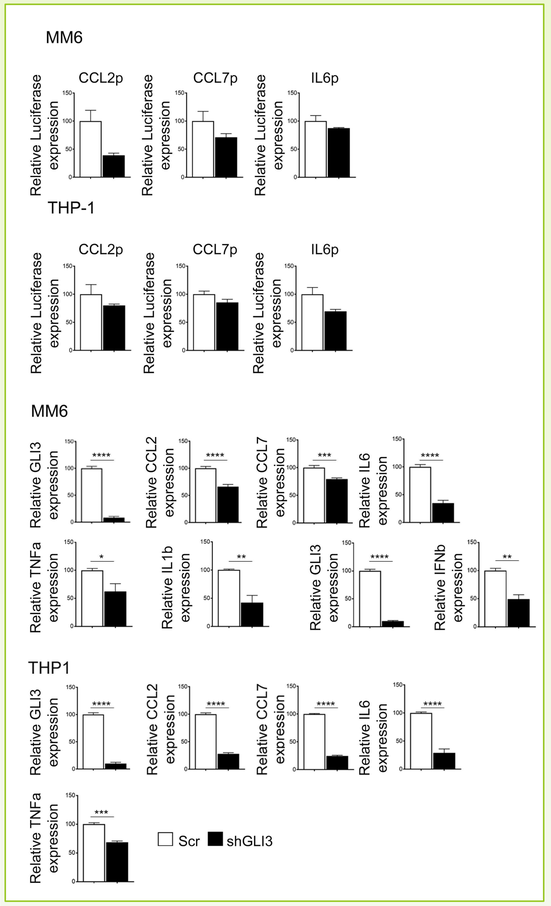
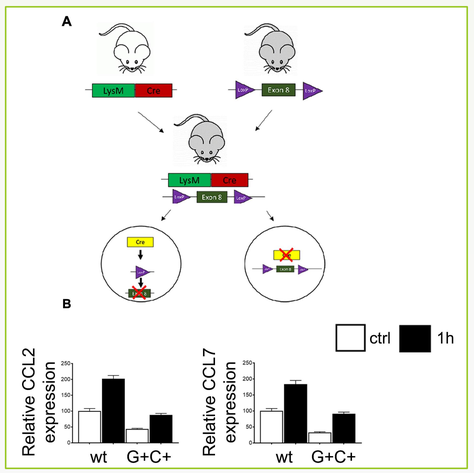


Figure 3: Top: Luciferase assay on monocytes transfected with indicated promotor constructs and RNAi targeting GLI3 (shGLI3) or Scrambled (Scr) control. **Bottom:** qPCR on monocytes transfected with shGLI3 or Scr for two days.

Future studies



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