

# Primary Intestinal Myofibroblasts Express the TNF-like Cytokine TL1A/TNFSF15 Following Stimulation with Pro-inflammatory Mediators

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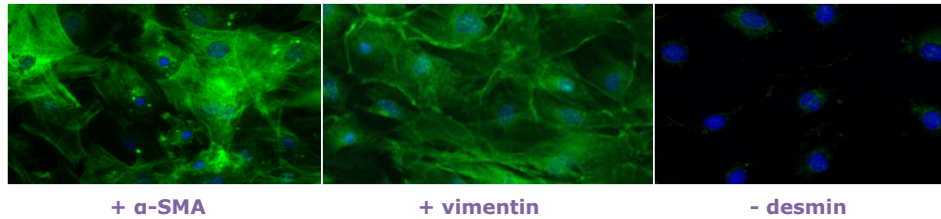
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**INTRODUCTION:** TL1A belongs to the TNF superfamily of cytokines (TNFSF15). It provides co-stimulatory signals for activated lymphocytes that express the functional receptor DR3. TL1A and DR3 are highly upregulated in intestinal areas with active IBD-related inflammation. Recently it was reported that transgenic mice with lymphoid- or myeloid-specific overexpression of TL1A develop colonic fibrosis (Bamias G. et al. *Curr Opin Gastroent* 2013, Bamias G. et al. *Dig Liver Dis*. 2012).

**AIM:** Our aim was to determine whether primary human intestinal myofibroblasts (IM) express TL1A under stimulation with IBD-associated pro-inflammatory cytokines.

**METHODS:** IM were isolated from endoscopically-obtained colonic biopsies from healthy controls (HC) and patients with Crohn's disease (CD), as previously described (Drygiannakis I, et al. *J Crohns Colitis*, 2013).

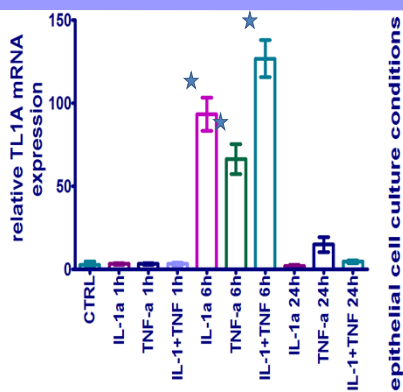
**Figure 1. IM Characterization**



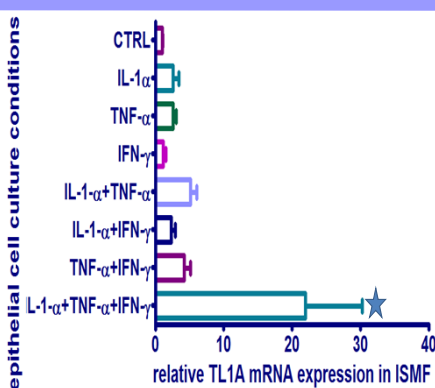
IM were cultured unstimulated or stimulated under various conditions: a. with rhTNF- $\alpha$  and/or rhIL-1 $\alpha$ ; b. with supernatants from colonic tissues obtained from HC and CD patients; and, c. with supernatants from epithelial HT-29-cell cultures which were unstimulated or stimulated with rhTNF- $\alpha$  and/or rhIL-1 $\alpha$  and/or rhIFN $\gamma$ . Total RNA was extracted from the cultured IM and the mRNA expression of TL1A and the receptor DR3 was measured by real time RT-PCR and immunofluorescence.

## RESULTS

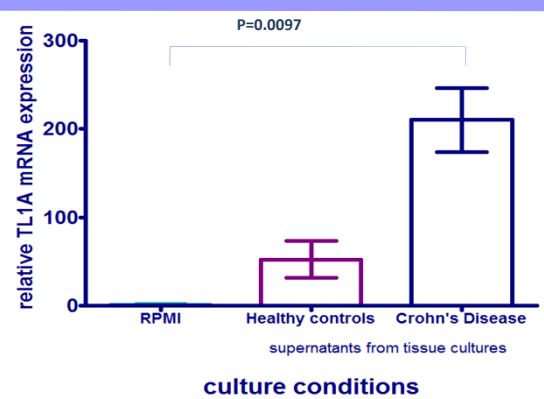
**Figure 2.**



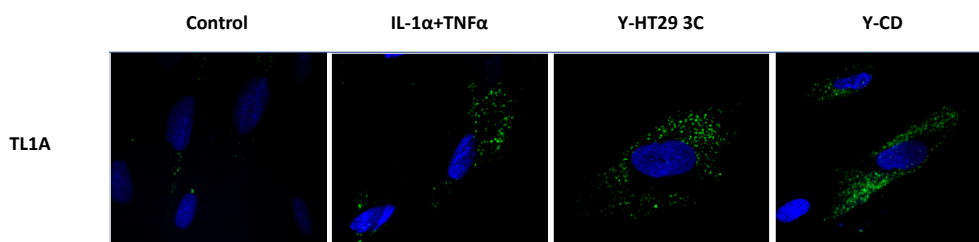
**Figure 3.**



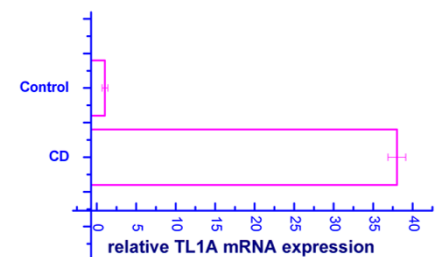
**Figure 4.**



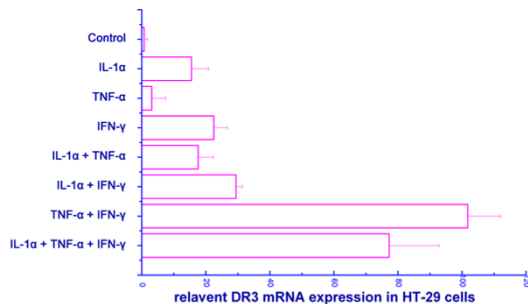
**Figure 5.**



**Figure 6.**



**Figure 7.**



**Figure 2. Pro-inflammatory cytokines IL-1 $\alpha$  and TNF- $\alpha$  induce TL1A overexpression in cultured IM.**

**Figure 3. Epithelial-derived soluble factors induce the expression of TL1A in cultured IM.**

**Figure 4. Soluble factors from intestinal tissue cultures of patients with Crohn's disease upregulate the expression of TL1A in cultured IM.**

**Figure 5. Inducible TL1A protein overexpression in IM pro-inflammatory factors.**

**Figure 6. TL1A expression in primary IM from HI and patients with active CD.**

**Figure 7. Pro-inflammatory cytokines induce the expression of DR3, the functional receptor for TL1A, in HT-29 cells.**

## CONCLUSIONS

Pro-inflammatory cytokines that are abundantly expressed in intestinal areas with active CD (TNF- $\alpha$ , IL-1 $\alpha$ , IFN- $\gamma$ ) induce the expression of the co-stimulatory cytokine TL1A in IM either directly or through the induction of epithelial-derived mediators. Our results raise the possibility that interactions between IM-derived TL1A and its receptor, DR3 on epithelial cells or lymphocytes may participate in the pathogenesis of chronic intestinal inflammation.