Pro-inflammatory stimuli do not induce IL-33 expression or secretion in colon epithelial cells

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Background: IL-33 is up-regulated in patients with ulcerative colitis (UC) and has been shown to play a protective role. Immunohistochemical analysis has shown that epithelial cells are a source of IL-33 in human UC biopsies; however, stimuli resulting in IL-33 secretion are still unknown. We hypothesized that pro-inflammatory stimuli would induce IL-33 expression and secretion in epithelial cells in vitro and that we would see increased IL-33 expression in epithelial cells from mice with colitis.

Methods: HT-29 and T-84 human colon cell lines were treated with TNF, IFN, or LPS for up to 72 hours and then pulsed with ATP for 30 minutes. Pre-pulse and post-pulse supernatants were analyzed with ELISA for IL-33 secretion. Lysed cells were analyzed with RT-PCR and Western blot. Colon mucosal cells were isolated from healthy IL-33 citrine reporter mice (IL-$33^{cit/+}$) mice and IL-$33^{cit/+}$IL-$10^{-/-}$ with colitis and analyzed by flow cytometry for IL-33 expression. E-Cadherin, CD45 and CD90 were used to distinguish between epithelial cells, immune cells and fibroblasts.

Results: Treatment of HT-29 and T-84 cells with TNF-α, IFN-y or LPS with or without ATP for 24, 48 or 72 hours did not increase IL-33 secretion, protein levels or gene expression. Epithelial cells and $CD45^+$ immune cells were not producing substantial amounts of IL-33 at baseline or during murine colitis. Fibroblasts were shown to be a source of IL-33 at baseline and during colitis, but a large fraction of the IL-33^+ cells still remain unidentified.

Conclusions: Although epithelial cells may express low levels of IL-33, we could not increase IL-33 expression or secretion in human colon cells with any of the pro-inflammatory stimuli we used. Furthermore, we did not see evidence of active IL-33 transcription in mouse colon epithelial cells at baseline or during colitis using IL-33 reporter mice. Future directions include using flow cytometry to discover other sources of IL-33 and then stimulating those cells to see if expression and/or secretion can be induced.

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