IL-13 and IL-4 Promote Proliferation and mRNA Expression of MUC2 and MUC5AC in Primary Human Conjunctival Goblet Cells

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Introduction

- Interleukin (IL-13) and IL-4 are signature type-2 cytokines involved in atopic dermatitis (AD).
- Monoclonal antibodies that inhibit signaling of these type-2 cytokines have demonstrated clinical efficacy in patients with moderate-severe atopic dermatitis (AD) [1-6].
- Increased incidence of conjunctivitis has been reported in AD patients after treatment with antibodies blocking IL-13 and IL-13 signaling [7-9].
- Dupilumab-induced conjunctivitis has been associated with goblet cell acetylated mucin deficiency as well as increased cell infiltration with increased number of Th2 cells [10-12], possibly due to Th1 polarization through inhibition of IL-4 signaling by dupilumab [13](Figure 2) [14-17].

Methods

- Primary human goblet cells were grown from cultured conjunctiva harvested from human donors and cleared from fibroblasts.
- Primary human conjunctival goblet cells were seeded in appropriately sized culture dishes and exposed to different concentrations of IL-13, IL-4, or IFN-γ or a combination thereof as indicated in the results panels. After 24, 48 or 72h incubation with the cytokines, cells were processed for cell counting by automated microscopy or for RNA extraction and qPCR analysis (Figure 4).

Results

Characterization of primary human CGCs

- Primary human goblet cells were cultured from cultured conjunctiva harvested from human donors and cleared from fibroblasts.
- Primary human conjunctival goblet cells were seeded in appropriately sized culture dishes and exposed to different concentrations of IL-13, IL-4, or IFN-γ or a combination thereof as indicated in the results panels. After 24, 48 or 72h incubation with the cytokines, cells were processed for cell counting by automated microscopy or for RNA extraction and qPCR analysis (Figure 4).
- In mouse and rat conjunctival goblet cells (CGC), IL-13 and IL-4 induce proliferation and mucin expression [18-20].
- IFN-γ has been shown to trigger the Unfolded Protein Response (UPR) in mouse CGCs (Figure 3).
- Chronic UPR signalling in CGCs leads to secretory dysfunction and eventually cell death [21].
- IFN-γ-mediated secretory dysfunction and CGC death contribute to dry eye diseases [22,23].
- The effect of IL-13 versus IL-4 on human CGCs has not been investigated.

Objective

- To investigate the effects of IL-13, IL-4 and IFN-γ on cell proliferation and mucin production in primary human CGCs.
- IL-13 and IL-4 induced expression of MUC2 and MUC5AC in primary human CGCs whereas IFN-γ induced expression of MUC5AC in primary human CGCs [24].
- Monoclonal antibodies that inhibit signaling of these type-2 cytokines have demonstrated clinical efficacy in patients with moderate-severe atopic dermatitis (AD) [1-6].
- Increased incidence of conjunctivitis has been reported in AD patients after treatment with antibodies blocking IL-13 and IL-13 signaling [7-9].
- Dupilumab-induced conjunctivitis has been associated with goblet cell acetylated mucin deficiency as well as increased cell infiltration with increased number of Th2 cells [10-12], possibly due to Th1 polarization through inhibition of IL-4 signaling by dupilumab [13] (Figure 2) [14-17].

Conclusions

- IFN-γ had a strong negative effect on primary human CGC proliferation and viability.
- IFN-γ, but not IL-13 and IL-4, triggered expression of Unfolded Protein Response markers in primary human CGCs, potentially directly impacting on cell health and protein (mucin) secretion.
- IL-13 and IL-4 showed functional redundancy by stimulating proliferation of primary human CGCs.
- IL-13 and IL-4 showed functional redundancy by increasing expression of MUC2 and MUC5AC mRNA in human CGCs.

References


Disclosures

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