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 **(Figure 1)**[1-3]
- Increased incidence of conjunctivitis has been reported in AD patients after treatment with antibodies blocking IL-4 and IL-13 signaling[1-5]
- Dupilumab-induced conjunctivitis has been associated with goblet cell scarcity and mucin deficiency as well as immune cell infiltrates with increased numbers of Th1 cells secreting IFN-γ, possibly due to Th1 polarization through inhibition of IL-4 signaling by dupilumab **(Figure 2)**[6-13]

Figure 1. Mode of action of dupilumab, tralokinumab, and lebrikizumab

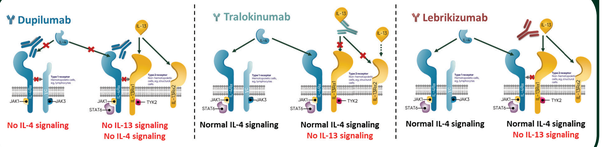
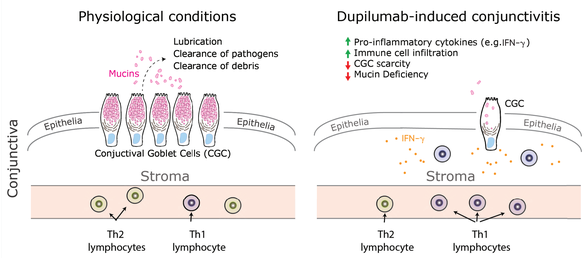


Figure 2. Schematic overview of the characteristics of dupilumab-induced conjunctivitis[6-13]

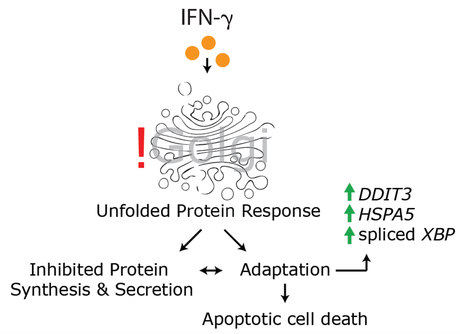


- In mouse and rat conjunctival goblet cells (CGC), IL-13 and IL-4 induce proliferation and mucin expression[14-17]
- IFN-γ has been shown to trigger the Unfolded Protein Response (UPR) in mouse CGCs **(Figure 3)**[17]
- Chronic UPR signaling in CGCs leads to secretory dysfunction and eventually cell death[17-21]
- IFN-γ–mediated secretory dysfunction and CGC death contribute to dry eye disease[17, 19-21]
- 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

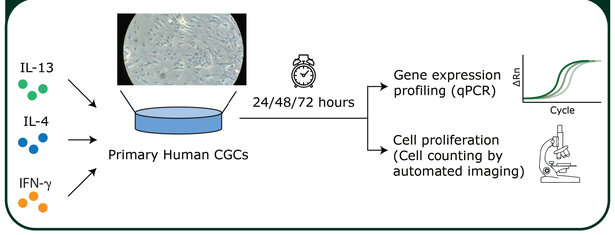
Figure 3. Overview of IFN-γ–induced unfolded protein response (UPR) pathway in mouse conjunctival goblet cells



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)**

Figure 4. Schematic representation of experimental set-up

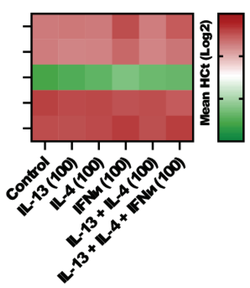


Results

Characterization of primary human CGCs

- Primary human CGCs expressed high levels of *IL4R, IL13RA1, IFNGR1* and *IFNGR2* mRNA **(Figure 5)**
- Primary human CGCs expressed very low levels of *IL13RA2* mRNA
- Little to no regulation of receptor expression in response to IL-13, IL-4 or IFN-γ

Figure 5. Expression of *IL4R, IL13RA1, IL13RA2, IFNGR1* and *IFNGR2* mRNA levels in primary human CGCs



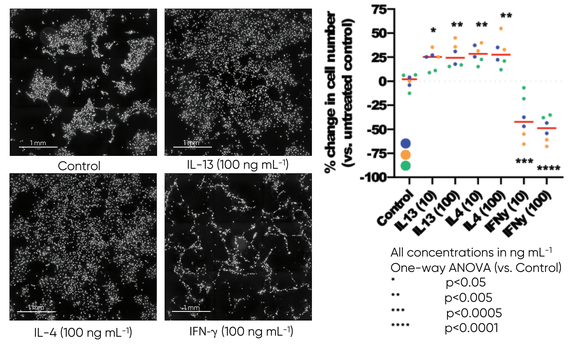
All concentrations in ng mL^{-1}
One-way ANOVA (vs. Control)
* p<0.05
** p<0.005
*** p<0.0005
**** p<0.0001
Empty non-significant

n=1 (measured in biological duplicate)

IL-13 and IL-4 promote CGC proliferation

- IL-13 and IL-4 both promoted proliferation of CGCs in a comparable manner **(Figure 6)**
- IFN-γ had a strong negative impact on CGC growth and viability
- These effects were observed in primary human CGCs derived from three independent donors and are both time- and dose-dependent (data not shown)

Figure 6. Effects of IL-13, IL-4 and IFN-γ on CGC proliferation measured by automated cell counting (images originate from donor 01)

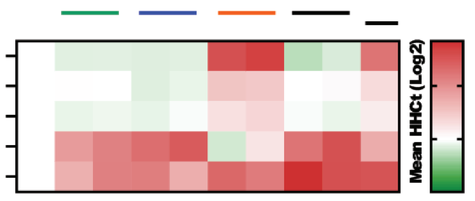


All concentrations in ng mL^{-1}
One-way ANOVA (vs. Control)
* p<0.05
** p<0.005
*** p<0.0005
**** p<0.0001

IL-13 and IL-4 induce expression of *MUC5AC* and *MUC2* mRNA in primary human CGCs whereas IFN-γ triggers the Unfolded Protein Response (UPR)

- Human CGCs expressed characteristic UPR markers upon IFN-γ stimulation, IL-13 and IL-4 did not induce this cellular stress response **(Figure 7)**
- *MUC5AC* is the major CGC secretory mucin in human tears to maintain surface hydration and clearance of pathogens and debris[21,22]
- Ocular *MUC5AC* deficiency has been observed in dupilumab-treated AD patients that developed conjunctivitis[8]
- *MUC2* is the major immune-regulatory product of small intestine resident GCs and is also expressed by CGCs[17,21,22]
- IL-13 and IL-4 both induced expression of *MUC2* and *MUC5AC* mRNA

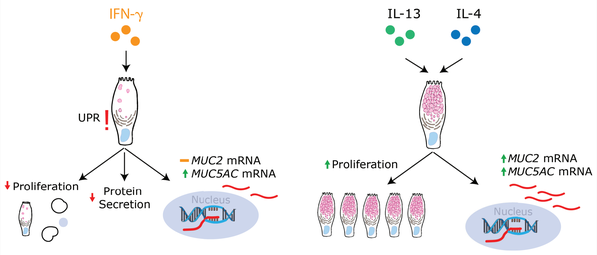
Figure 7. Increased expression of *MUC2* and *MUC5AC* mRNA in response to IL-13 and IL-4, and IFN-γ–mediated induction of the UPR in primary human CGCs



All concentrations in ng mL^{-1}
One-way ANOVA (vs. Control)
* p<0.05
** p<0.005
*** p<0.0005
**** p<0.0001
Empty non-significant

Data from one representative experiment
MUC2: n = 4
MUC5AC: n = 1

Figure 8. Summary of the effects of IL-13, IL-4 and IFN-γ on primary human CGCs



Due to the **functional redundancy** between **IL-13** and **IL-4**, targeted treatment with monoclonal antibodies that **specifically neutralize IL-13**, might be associated with **a lower incidence/severity of conjunctivitis** in AD patients, compared to inhibiting both IL-13 and IL-4.

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 primary human CGCs

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Disclosures

Pernille Rævdal has received funding from LEO Pharma. **Anne Hedengran Nagstrup** acted as speaker for Laboratories THEA. **Steffen Heegaard** has attended advisory boards for Sanofi-Genzyme and received speaker honoraria from LEO Pharma, Sanofi-Genzyme, Santen and Thea pharmaceuticals. **Jacob Thyssen** is speaker/advisor/investigator for Abbvie, Pfizer, LEO Pharma, Sanofi-Genzyme, Regeneron, Almirall, and Eli Lilly & Co. **Miriam Kolko** has received funding from LEO Pharma and Thea Pharmaceuticals, received speaker honoraria from Santen and Thea Pharmaceuticals, attends advisory boards for Allergan and Santen, and is a consultant for Thea Pharmaceuticals. **Maxim Tollenaere, Mads Roepke, Shannon Schneider,** and **Hanne Norsgaard** are employees of LEO Pharma.

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