

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

Maxim A.X. Tollenaere,<sup>1</sup> Pernille Rævdal,<sup>2,3</sup> Steffen Nagstrup,<sup>2,3</sup> Anne Heegaard,<sup>3</sup> Mads Roepke,<sup>1</sup> Shannon Schneider,<sup>4</sup> Hanne Norsgaard,<sup>1</sup> Jacob P. Thyssen,<sup>5</sup> Miriam Kolko<sup>2,3</sup>

<sup>1</sup>LEO Pharma A/S, Ballerup, Denmark; <sup>2</sup>Department of Drug Design and Pharmacology, University of Copenhagen, Denmark; <sup>3</sup>Department of Ophthalmology, University Hospital of Copenhagen, Denmark; <sup>4</sup>LEO Pharma, Madison, New Jersey, USA; <sup>5</sup>Department of Dermatology, Frederiksberg and Bispebjerg Hospital, Denmark

**Introduction.** Interleukin (IL)-13 and IL-4 are key cytokines involved in the pathogenesis of atopic dermatitis (AD). Monoclonal antibodies that inhibit signaling of these type-2 cytokines have demonstrated clinical efficacy in AD patients with moderate-to-severe disease. Examples of these are dupilumab, which targets IL-4R $\alpha$  thereby inhibiting IL-13 and IL-4 signaling, as well as tralokinumab and lebrikizumab, which specifically neutralize IL-13. However, these treatments have also been associated with an increased incidence of conjunctivitis. Conjunctival goblet cell scarcity and mucin deficiency have been reported in AD patients treated with dupilumab, which may explain partly the conjunctivitis observed. Importantly, the impact of IL-13 versus IL-4 on human conjunctival goblet cells is not fully understood. Inhibition of IL-4 may induce Th1 polarization with increased production of interferon gamma (IFN- $\gamma$ ), which has been shown to lead to secretory dysfunction of mucins and to trigger conjunctival goblet cell apoptosis.

**Methods.** In this mechanistic study, we explored the impact of the type-2 cytokines IL-13 and IL-4 as well as that of the type-1 cytokine IFN- $\gamma$  on primary human conjunctival goblet cells in vitro.

**Results.** IL-13 and IL-4 both promoted proliferation of primary human conjunctival goblet cells in a dose- and time-dependent manner, whereas IFN- $\gamma$  had a strong negative impact on conjunctival goblet cell growth and viability. In addition, IL-13 and IL-4 both led to increased gene expression of two key mucins, MUC2 and MUC5AC, in primary human conjunctival goblet cells. In summary, IL-13 and IL-4 can both act as regulators of human conjunctival goblet cell proliferation and mucin expression. As conjunctival goblet cells are essential for maintaining homeostasis of the conjunctival mucosal surface, our findings may at least in part provide a mechanistic explanation behind the ophthalmological adverse events observed after treatment with biologics inhibiting IL-4 and IL-13 signaling.

**Conclusion.** Due to the functional redundancy of IL-13 and IL-4 on conjunctival goblet cell biology, targeted treatment with monoclonal antibodies that specifically neutralize IL-13 might be associated with a lower incidence of conjunctivitis in AD patients compared to inhibiting both IL-13 and IL-4 with dupilumab.