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SUPPORTING INFORMATION

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IL-4 and IL-13 both contribute to the homeostasis of human conjunctival goblet cells in vitro

To the Editor,

Interleukin (IL)-13 and IL-4 are key cytokines in atopic dermatitis (AD) pathogenesis. Monoclonal antibodies inhibiting signaling of these type 2 cytokines have demonstrated clinical efficacy in moderate-to-severe AD patients.[1] Examples include dupilumab, which targets IL-4Rα, inhibiting IL-13 and IL-4 signaling, and tralokinumab and lebrikizumab, which specifically neutralize IL-13. These treatments have been associated with increased conjunctivitis and blepharitis in AD patients; manifestations that are regarded to be part of the AD syndrome.[2]

Conjunctival goblet cell (CGC) scarcity, mucin deficiency, and immune cell infiltrates with increased numbers of Th1 cells secreting interferon-gamma (IFN-γ), have been reported in AD patients that developed conjunctivitis upon dupilumab treatment.[3,4] Inhibition of IL-4 signaling by dupilumab may induce Th1 polarization with increased IFN-γ production, leading to secretory dysfunction of mucins and triggering CGC apoptosis.[4,5]

Mouse and rat CGC cultures are highly sensitive to immunomodulatory mediators, including IL-13, IL-4, and IFN-γ, which directly impact cell proliferation and mucin secretion.[5] The effects of these cytokines on human CGCs are not well understood. We isolated primary CGCs from cultured conjunctiva from human donors, after ethical approval and informed consent (detailed in Data S1), and assessed cell proliferation by image-based cell counting and mucin expression by qPCR in response to the aforementioned cytokines (Figure 1A). We confirmed that human CGCs expressed the relevant receptors for IL-13, IL-4, and IFN-γ signaling (Figure 1B). Next, we assessed the cytokines' effect on CGC proliferation using cells from three human donors. IL-13 and IL-4 promoted CGC cell proliferation comparably, whereas IFN-γ had a strong negative impact on cell proliferation and viability (Figure 1C and Figure S1A). Dose- and time-dependent trends were observed (Figure 1D). In murine models, IFN-γ triggers the unfolded protein response (UPR) in CGCs. This pathway is associated with secretory dysfunction and

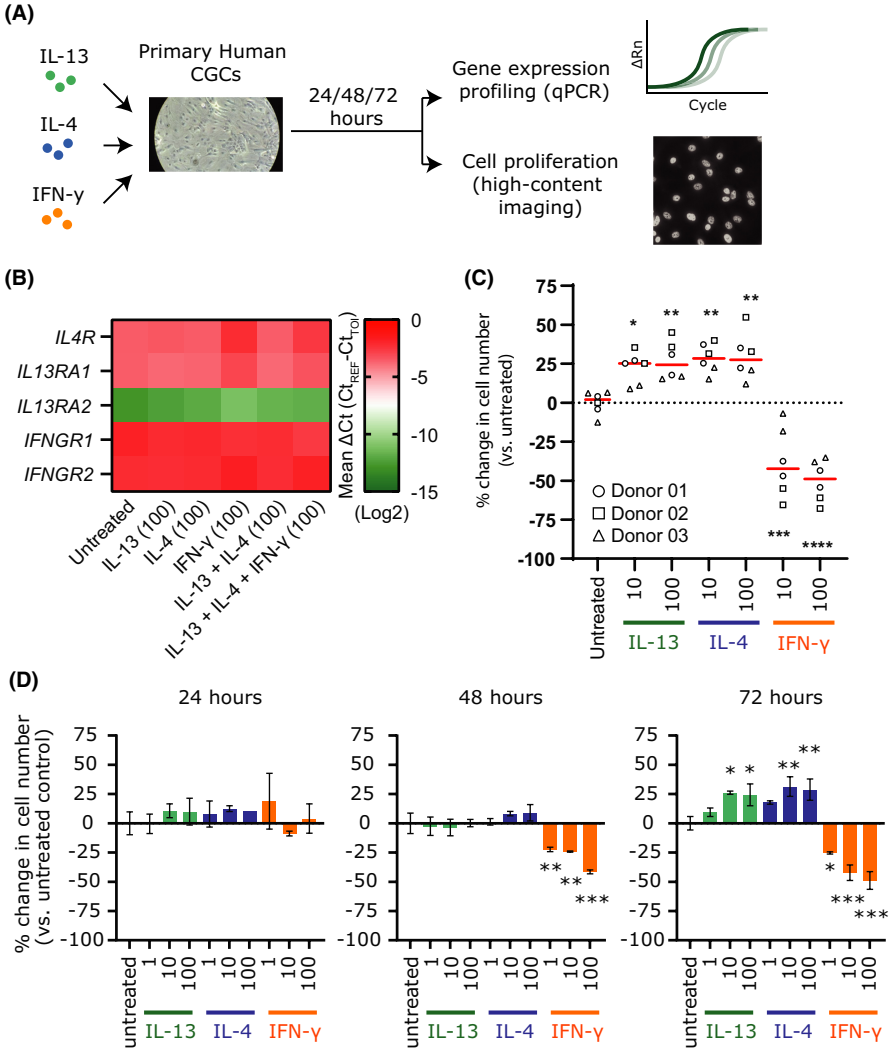

FIGURE 1 IL-13 and IL-4 promote conjunctival goblet cell proliferation. (A) Experimental design. (B) mRNA expression of interleukin (IL)-13, IL-4, and interferon-gamma (IFN-γ) signaling receptors in primary human conjunctival goblet cells (CGCs) measured by qPCR. (C) Change in number of CGCs from three donors after 72 h of incubation with indicated cytokines. Red bars indicate mean value per condition. (D) Proliferation of CGCs from donor #1 after 24, 48, and 72 h of incubation with indicated cytokines (mean ± SD). All concentrations shown are in ng/ml. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$, and $^{****}p < 0.0001$

CGC death, which may contribute to development of dry eye disease.[5] To investigate if this response was also activated by IFN-γ in human CGCs, we assessed the expression of several UPR markers. We detected upregulation of UPR markers (*DDIT3/CHOP*, spliced *XBP1*, and *HSPA5/GRP78*) in human CGCs in response to IFN-γ, but not IL-13 or IL-4 (Figure 2A and Figure S2A–E), indicating that IFN-γ triggers the same cellular stress response in human CGCs as in murine models. Finally, our analysis revealed that IL-13 and IL-4 both lead to increased mRNA expression of two key ocular mucins, *MUC2* and *MUC5AC* in human CGCs. *MUC5AC* deficiency in tear fluid has been observed in AD patients with dupilumab-induced conjunctivitis.[6] Comparable to murine models, IFN-γ also led to increased expression of *MUC5AC*. It has, however, been demonstrated that while IFN-γ increases *MUC5AC* transcripts, IFN-γ-induced UPR signaling causes secretory dysfunction and inhibits *MUC5AC* secretion from CGCs.[5]

While in vitro CGC cultures are appropriate to investigate molecular mechanisms, the model system in this study lacks the cellular complexity of the conjunctival tissue and associated immune cells. Also, cultures of isolated CGCs showed considerable variation in baseline expression of mucins and proliferative potential between donors. Nevertheless, our results show that IFN-γ both reduces proliferation and viability of human CGCs and activates UPR signaling, which suggests a limitation in their capacity to produce and/or secrete mucins (Figure 2B). IL-13 and IL-4 on the other hand showed functional redundancy by both being able to stimulate proliferation and expression of *MUC2* and *MUC5AC* mRNA in primary human CGCs (Figure 2C). As CGCs are essential for maintaining homeostasis of the conjunctival mucosal surface, our findings may in part provide a mechanistic explanation behind the ocular adverse events observed after treatment with biologics inhibiting IL-4 and IL-13 signaling. Due to the functional redundancy of IL-13

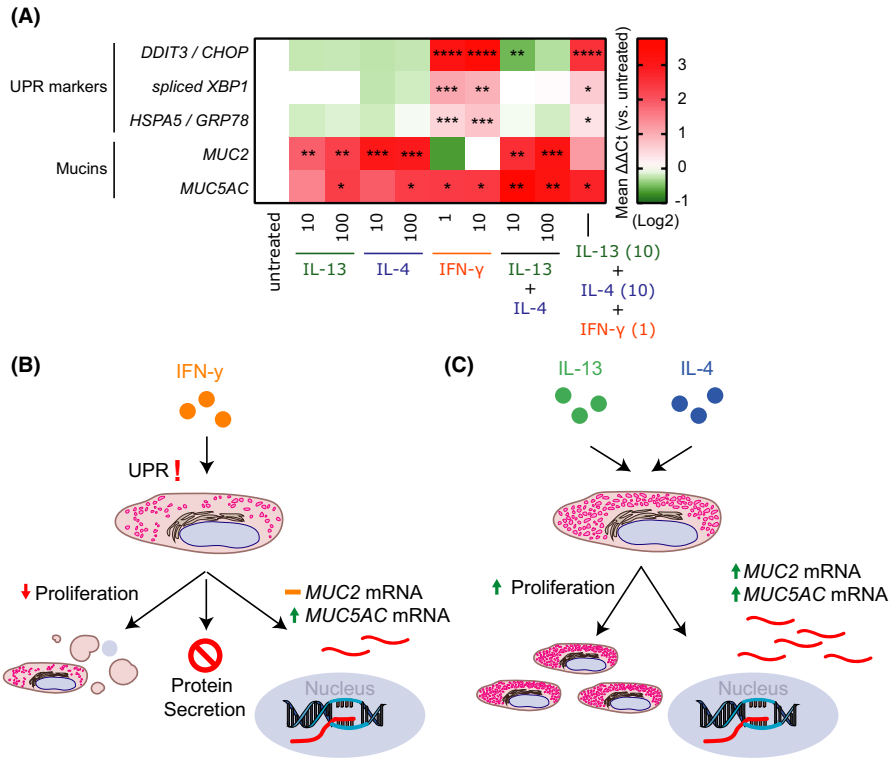

FIGURE 2 Expression of unfolded protein response and mucin genes in primary human conjunctival goblet cells. (A) mRNA expression of unfolded protein response (UPR) markers and mucins in primary human conjunctival goblet cells (CGC) upon incubation with the indicated cytokines. Change in gene expression is visualized as ΔΔCt value compared with untreated control. (B) Effects of interferon-gamma (IFN-γ) on primary human CGCs. (C) Functional redundant effects of IL-13 and IL-4 on primary human CGC proliferation and mucin mRNA expression. All concentrations shown are in ng/ml. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.005$

and IL-4, the data presented here might explain why targeted treatment neutralizing only IL-13 is associated with a lower incidence and severity of conjunctivitis compared with inhibiting both IL-13 and IL-4 signaling.[7]

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AUTHOR CONTRIBUTIONS

P.H., M.T., and A.H. performed experiments. M.T. performed formal data analysis and visualization. S.H., P.A., M.R., J.T., M.K., and H.N. were involved in conceptualization. M.T. and H.N. wrote the manuscript. All authors reviewed and approved the manuscript.

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SUPPORTING INFORMATION

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COVID-19 in the absence of eosinophils: The outcome of confirmed SARS-CoV-2 infection whilst on treatment with benralizumab

To the Editor,

Patients with severe asthma have been reported to experience more severe COVID-19 disease outcomes including hospital admission, ICU admission and death.[1] Blood eosinopenia was one of the earliest reported findings in hospitalized patients with COVID-19, opening discussion as to whether eosinophils may have an anti-viral or deleterious role in the immune response against SARS-CoV-2 infection.[2] A number of predominantly pre-clinical studies have suggested a role for eosinophils in anti-viral immunity. For example, eosinophils express several endosomal Toll-like receptors (TLRs) including TLR7 which has been shown to enable eosinophils to recognize single-stranded RNA viruses including coronavirus.[3]

Benralizumab is an anti-IL5R monoclonal antibody licensed for the treatment of severe eosinophilic asthma (SEA), resulting in the near-complete depletion of blood and tissue eosinophils.[4,5] To date, a critical role for the eosinophil in anti-viral defence has been called into question by the absence of safety signals relating to viral infections in the large phase 3 and extension trials of benralizumab in subjects with asthma and COPD.[6,7] However, little is known about the outcomes of SARS-CoV-2 infection in individuals with benralizumab-induced eosinopenia.

We identified patients with SEA established on treatment with benralizumab (treatment commenced prior to April 2021) at Guy's Severe Asthma Centre, London, UK, and contacted them by telephone throughout May and June 2021 to establish whether they had experienced a confirmed PCR-positive SARS-CoV-2 infection since commencing benralizumab. Clinical and demographic characteristics were recorded along with the outcome of infection, including the need for hospitalization and intensive care admission. Patients with severe COVID-19 as defined by the requirement for hospitalization were compared to those experiencing non-severe infections treated in the community. Approval to report observational data for this cohort was granted by the GSTT ethics committee (ref 11999).

Two hundred and sixty-eight patients on treatment with benralizumab were contacted with 24/268 (9%) reporting a confirmed infection with a positive PCR test for SARS-CoV-2. Of 24, 18 (75%) reported non-severe self-limiting infections with the remaining 6 (25%) experiencing more severe infections requiring hospitalization (median length of stay 6 [IQR 1–8] days). No patients required admission to intensive care or needed mechanical ventilation. Of 24, 22 patients in this cohort were unvaccinated at the time of infection. The remaining 2/24 (8.3%) had received a single dose of SARS-CoV-2 vaccine (in both cases Oxford/Astra Zeneca) at least 2 weeks prior to confirmed infection. Of 24, 23 patients had received at least 6 months' treatment with benralizumab at the time of COVID-19 infection. One patient had received 3 doses (at least 8 weeks' treatment).