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## IL-10, TGF- $\beta$ and glucocorticoid prevent the production of type 2 cytokines in human group 2 innate lymphoid cells

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### Keywords

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### To the Editor

Allergic diseases are characterized by accumulation and activation of inflammatory cells including Th2 cells and mast cells. Recent studies indicate that group 2 innate lymphoid cells (ILC2s) also produce a large quantity of type 2 cytokines, and have pathogenic roles in allergic and type 2 inflammatory diseases including allergic asthma and chronic rhinosinusitis with nasal polyps (CRSwNP).<sup>1–3</sup> Although the mechanisms of suppression of Th2 cells and mast cells have been extensively investigated, there is only minimal data on

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corresponding mechanisms of suppression for ILC2s in type 2 inflammation. Studies in mice indicate that immunosuppressive cytokines, IL-10 and TGF- $\beta$ , and glucocorticoids suppress ILC2-mediated type 2 inflammation.<sup>4–6</sup> However, the role of IL-10 and TGF- $\beta$  in human ILC2s is still unclear and only mRNA expression of the IL-10 receptor has been reported.<sup>4</sup> Although Walford *et al.* reported that the frequency of ILC2 in nasal polyps (NPs) was reduced by treatment with systemic glucocorticoid,<sup>6</sup> two other groups showed that ILC2s were resistant to glucocorticoids especially in patients with asthma.<sup>7, 8</sup> However, both studies examined the role of glucocorticoids in ILC2s using PBMC, which are a mixture of cells, and identified the presence of intracellular cytokine<sup>+</sup> ILC2s by flow cytometry after activation.<sup>7, 8</sup> Therefore the direct effect of glucocorticoid on human ILC2s is also still unclear.

To clarify the role of IL-10 and TGF- $\beta$  in human ILC2, we identified ILC2s by flow cytometry (see Fig. E1 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)),<sup>3</sup> and investigated the presence of receptors for IL-10 and TGF- $\beta$  on human ILC2s in peripheral blood, tonsil (a model of lymphoid tissue) and NP (a model of type 2 inflammatory disease). Detailed methods for experiments, our study population (see Table E1) and supplemental figures E1–E6 are given in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org). We found that IL-10 receptor subunits, IL-10RA and IL-10RB, and a TGF- $\beta$  type II receptor (TGFBR2) were expressed on ILC2s from blood, tonsil and NP (Fig. 1AB, see Fig. E2 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) and not shown). Levels of IL-10RA and TGFBR2 on ILC2 were similar to Th2 cells which are well known to react with IL-10 and TGF- $\beta$  (see Fig. E3 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). Although we detected the IL-10 receptor complex, the level of protein expression on blood ILC2s was not high based on flow cytometry (Fig. 1A and see Fig. E2B). We next investigated whether IL-10 was able to induce phosphorylation of STAT3 in blood ILC2s since IL-10 is a well known activator of STAT3. We found that IL-10 dose-dependently and significantly induced the phosphorylation of STAT3 in blood ILC2s (Fig. 1C). This result suggests that functional IL-10 receptor complex is present on human ILC2s.

To investigate the functional role of IL-10 and TGF- $\beta$  in ILC2s, we examined whether IL-10 and TGF- $\beta$  suppressed activation of ILC2s sorted from human blood. IL-33 is known to induce morphological changes in ILC2s and induces the production of type 2 cytokines.<sup>1, 2</sup> We therefore incubated the sorted blood ILC2s with IL-33 and TSLP in the presence or absence of IL-10 or TGF- $\beta$ 1 for 4 days and examined the morphology of ILC2s by cytospin and production of type 2 cytokines from ILC2s by Luminex. We found that the morphological changes to ILC2s induced by IL-33+TSLP were almost completely blocked by IL-10 but not by TGF- $\beta$  (Fig. 2A). We found that IL-10 significantly suppressed IL-33- and IL-33+TSLP-mediated production of IL-4, IL-5, IL-9 and IL-13 in blood ILC2s (Fig. 2B and see Fig. E4A in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). We also found that TGF- $\beta$ 1 significantly suppressed IL-33- and IL-33+TSLP-mediated production of IL-4, IL-5 and IL-13 but significantly enhanced production of IL-9 in human blood ILC2s (Fig. 2C and see Fig. E4B). These results suggest that IL-10 and TGF- $\beta$  suppress the activation of ILC2s in humans, although the effect of TGF- $\beta$  may be weaker than IL-10.

To examine the role of glucocorticoids, we incubated sorted human blood ILC2s with IL-33 and TSLP in the presence or absence of 0.01% DMSO (vehicle control) or 100 nM dexamethasone for 4 days. We found that dexamethasone suppressed the morphological changes to ILC2s and the production of IL-4, IL-5, IL-9 and IL-13 induced by IL-33+TSLP in blood ILC2s (Fig. 2AD). To confirm whether the dexamethasone mediated inhibition was via glucocorticoid receptors (GRs), we added a GR antagonist, RU-486 (100 nM), together with dexamethasone. We found that RU-486 significantly inhibited dexamethasone-mediated suppression of type 2 cytokine production in ILC2s (see Fig E5 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org)). In contrast to our result (Fig. 2D), Liu *et al.* reported that glucocorticoid could not suppress IL-33-mediated activation of human ILC2s in the presence of TSLP using PBMC.<sup>8</sup> This discrepancy suggests that TSLP and/or IL-33 may also act directly and/or indirectly on other immune cells present in PBMCs and factors from these other immune cells may cancel the glucocorticoid-mediated suppressive effect on ILC2s when cultured together. Future studies will be required to identify these factors.

Although we found that IL-10, TGF- $\beta$ 1 and dexamethasone inhibited the function of human ILC2s, the mechanism and degree of inhibition by these factors might be different. We found that only dexamethasone induced apoptosis and cell death in human blood ILC2s (not shown). We also found that the inhibitory effect of TGF- $\beta$  may be weaker than IL-10 and glucocorticoids, and that TGF- $\beta$ 1 enhanced IL-33-mediated production of IL-9 in human ILC2. These results suggest that production of IL-9 is differentially regulated in ILC2s compared to the classical Th2 cytokines IL-4, IL-5 and IL-13. Future study will be required to identify the mechanisms of suppression for each inhibitor.

Recent studies showed that peripheral blood ILC2s were increased during pollen season and that this seasonal increase was abrogated in seasonal allergic rhinitis patients who received grass pollen immunotherapy.<sup>9</sup> Successful immunotherapy was associated with the induction of Treg cells that produce IL-10 and TGF- $\beta$ .<sup>9</sup> Our current study showed that IL-10 and TGF- $\beta$ 1 potently suppressed the activation of ILC2s (Fig. 2). This suggests that immunotherapy may not only suppress Th2 cell-mediated reactions but also inhibit ILC2-mediated inflammation via induction of Treg-mediated cytokines.

Since allergic diseases are characterized by chronic inflammation, it is therefore important to ask whether inhibitory factors can also suppress previously activated ILC2. We recently found that NP ILC2s were already activated in NP *in vivo*, and sorted NP ILC2s but not blood ILC2s spontaneously released type 2 cytokines without additional stimuli.<sup>3</sup> Interestingly, IL-10, TGF- $\beta$ 1 and dexamethasone suppressed this spontaneous production of IL-5 and IL-13 in NP ILC2s within a small cohort (see Fig. E6 in this article's Online Repository at [www.jacionline.org](http://www.jacionline.org) and not shown). This result suggests that these inhibitors may reduce the ongoing ILC2-mediated inflammation that is found in chronic type 2 inflammatory diseases. However, it will require a larger study to confirm the current findings in NP and other diseases.

In conclusion, we report here that functional receptors for IL-10, TGF- $\beta$  and glucocorticoids are expressed on human ILC2s and IL-10, TGF- $\beta$  and glucocorticoids strongly suppress the activation of human ILC2s. Our data suggests that induction of allergen-specific Tregs,

IL-10 and TGF- $\beta$  and treatment with glucocorticoids would have strong benefits in allergic and type 2 inflammatory diseases by virtue of suppressing local T cells, mast cells and ILC2s.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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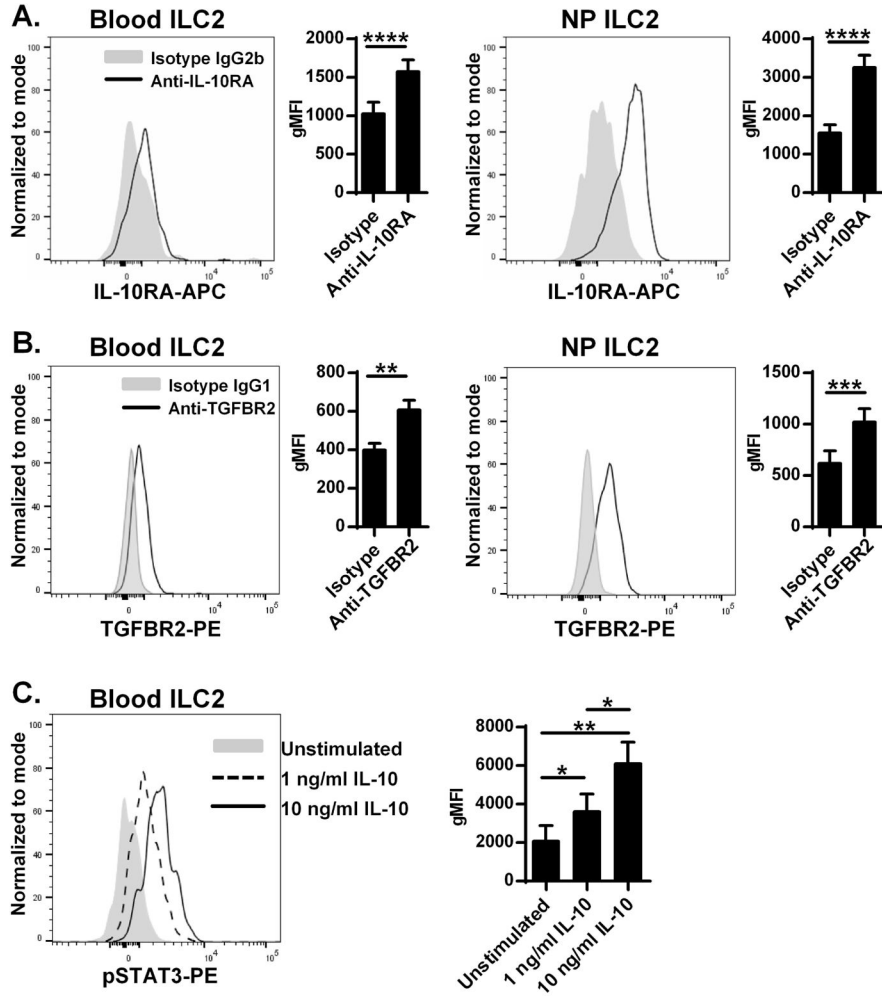
## Abbreviations

<b>GR</b>	Glucocorticoid receptor
<b>CRS</b>	Chronic rhinosinusitis
<b>CRSwNP</b>	CRS with nasal polyps
<b>ILC</b>	Innate lymphoid cell
<b>ILC2</b>	Group 2 innate lymphoid cell
<b>NP</b>	Nasal polyp
<b>TSLP</b>	Thymic stromal lymphopoietin
<b>Treg</b>	Regulatory T cell

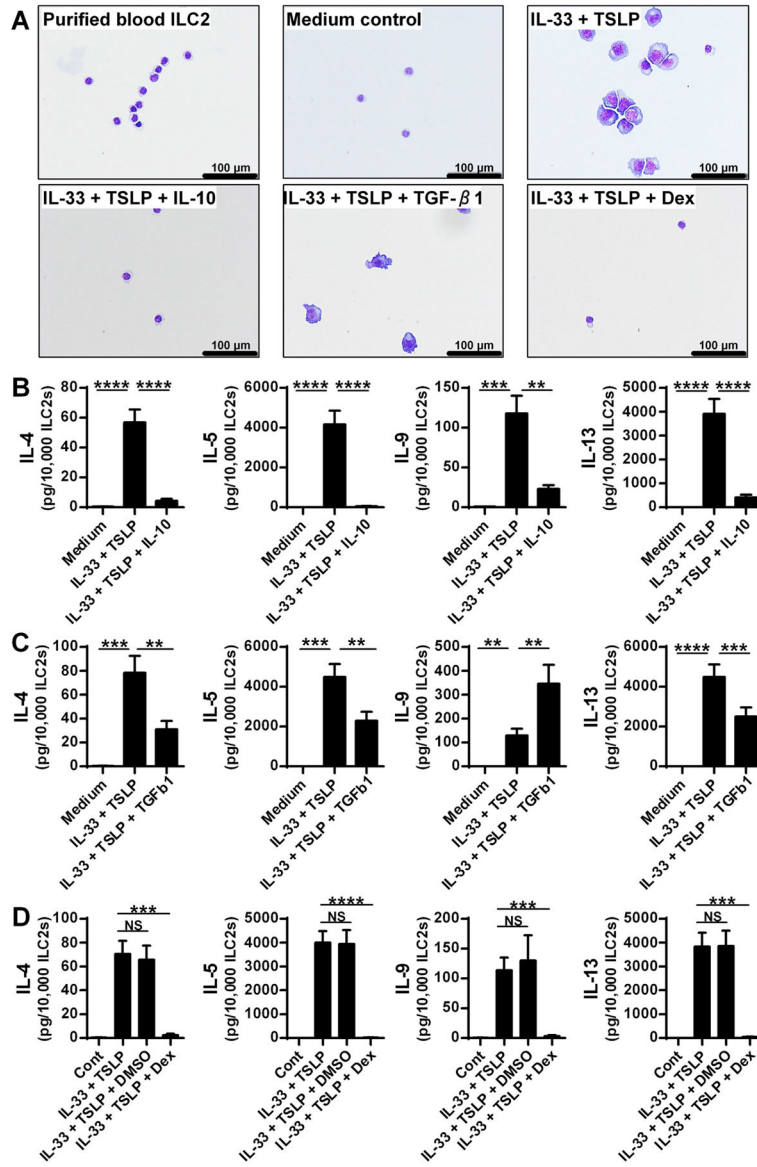
## References

1. Mjosberg JM, Trifari S, Crellin NK, Peters CP, van Drunen CM, Piet B, et al. Human IL-25- and IL-33-responsive type 2 innate lymphoid cells are defined by expression of CCR4 and CD161. *Nat Immunol.* 2011; 12:1055–62. [PubMed: 21909091]
2. Bartemes KR, Kephart GM, Fox SJ, Kita H. Enhanced innate type 2 immune response in peripheral blood from patients with asthma. *J Allergy Clin Immunol.* 2014; 134:671–8. e4. [PubMed: 25171868]
3. Puposki JA, Klingler AI, Tan BK, Soroosh P, Banie H, Lewis G, et al. Group 2 innate lymphoid cells are elevated and activated in chronic rhinosinusitis with nasal polyps. *Immun Inflamm Dis.* 2017 Apr 19. Epub ahead of print. doi: 10.1002/iid3.161
4. Morita H, Arae K, Unno H, Miyauchi K, Toyama S, Nambu A, et al. An Interleukin-33-Mast Cell-Interleukin-2 Axis Suppresses Papain-Induced Allergic Inflammation by Promoting Regulatory T Cell Numbers. *Immunity.* 2015; 43:175–86. [PubMed: 26200013]

5. Rigas D, Lewis G, Aron JL, Wang B, Banie H, Sankaranarayanan I, et al. Type 2 innate lymphoid cell suppression by regulatory T cells attenuates airway hyperreactivity and requires inducible T-cell costimulator-inducible T-cell costimulator ligand interaction. *J Allergy Clin Immunol.* 2017; 139:1468–77. e2. [PubMed: 27717665]
6. Walford HH, Lund SJ, Baum RE, White AA, Bergeron CM, Husseman J, et al. Increased ILC2s in the eosinophilic nasal polyp endotype are associated with corticosteroid responsiveness. *Clin Immunol.* 2014; 155:126–35. [PubMed: 25236785]
7. Jia Y, Fang X, Zhu X, Bai C, Zhu L, Jin M, et al. IL-13+ Type 2 Innate Lymphoid Cells Correlate with Asthma Control Status and Treatment Response. *Am J Respir Cell Mol Biol.* 2016; 55:675–83. [PubMed: 27314535]
8. Liu S, Verma M, Michalec L, Liu W, Sripada A, Rollins D, et al. Steroid resistance of airway type 2 innate lymphoid cells from patients with severe asthma: The role of thymic stromal lymphopoietin. *J Allergy Clin Immunol.* 2017 Apr 20. pii: S0091-6749(17)30660–7. Epub ahead of print. doi: 10.1016/j.jaci.2017.03.032
9. Kouser L, Kappen J, Walton RP, Shamji MH. Update on Biomarkers to Monitor Clinical Efficacy Response During and Post Treatment in Allergen Immunotherapy. *Curr Treat Options Allergy.* 2017; 4:43–53. [PubMed: 28413769]



**Figure 1. Presence of receptors for IL-10 and TGF- $\beta$  on human ILC2**  
 Representative histograms of flow cytometric plots for IL-10RA (A) and TGFBR2 (B) in ILC2s from a blood sample and a NP are shown. Levels of cell surface expression of receptors on ILC2s from blood (n=10) and NPs (n=10) are shown by geometric mean fluorescence intensity (gMFI). PBMC were stimulated with medium control (filled), 1 (dashed line) or 10 (solid) ng/ml IL-10 for 15 minutes. The level of intracellular phospho-STAT3 in blood ILC2s was detected by flow cytometry (C, n=6). \* p<0.05, \*\* p<0.01, \*\*\*\* p<0.0001, by the Paired *t* test (A and B) and one-way ANOVA (C).



**Figure 2. IL-10, TGF-β and dexamethasone inhibit activation of human ILC2**  
 Sorted blood ILC2s were cultured with 10 ng/ml IL-33 and 10 ng/ml TSLP in the presence or absence of 10 ng/ml IL-10 (B, n=8), 20 ng/ml TGF-β1 (C, n=6), 0.01% DMSO (vehicle control) and 100 nM dexamethasone (Dex) (D, n=6) for 4 days. The morphology of ILC2s was examined by cytopsin with Diff-Quik staining (A). The concentrations of IL-4, IL-5, IL-9 and IL-13 were measured by using Luminex (B–D). \* p<0.05, \*\* p<0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001, by one-way ANOVA.