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ORIGINAL ARTICLE

Basic and Translational Allergy Immunology

IL-37 regulates allergic inflammation by counterbalancing pro-inflammatory IL-1 and IL-33

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Abstract

Background: Children with asthma have impaired production of interleukin (IL) 37; in mice, IL-37 reduces hallmarks of experimental allergic asthma (EAA). However, it remains unclear how IL-37 exerts its inhibitory properties in asthma. This study aimed to identify the mechanism(s) by which IL-37 controls allergic inflammation.

Methods: IL-37 target cells were identified by single-cell RNA-seq of IL-1R5 and IL-1R8. Airway tissues were isolated by laser-capture microdissection and examined by microarray-based gene expression analysis. Mononuclear cells (MNC) and airway epithelial cells (AECs) were isolated and stimulated with allergen, IL-1β, or IL-33 together with recombinant human (rh) IL-37. Wild-type, IL-1R1– and IL-33–deficient mice with EAA were treated with rhIL-37. IL-1β, IL-33, and IL-37 levels were determined in sputum and nasal secretions from adult asthma patients without glucocorticoid therapy.

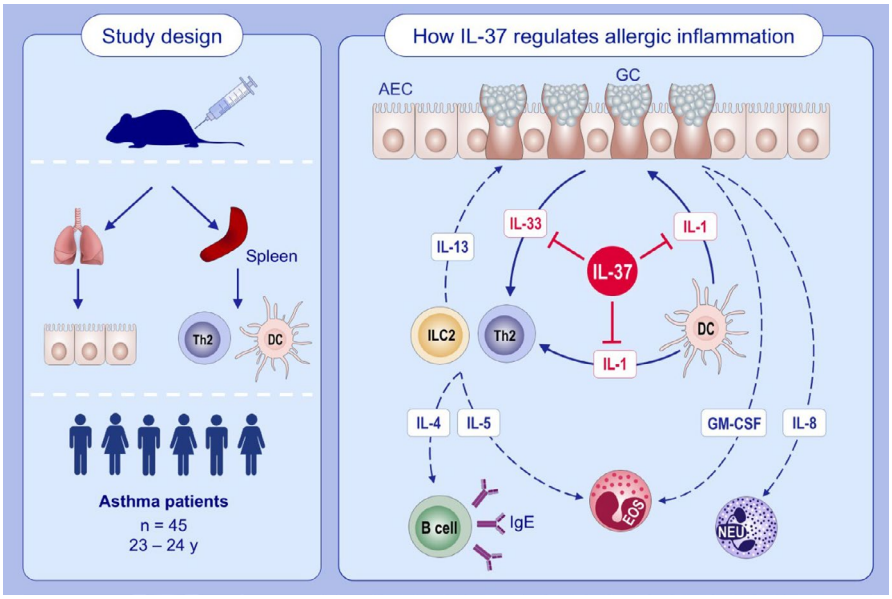
Results: IL-37 target cells included AECs, T cells, and dendritic cells. In mice with EAA, rhIL-37 led to differential expression of >90 genes induced by IL-1β and IL-33.

Alexandra Schröder and Lars P. Lunding equal contribution to this work.

rhIL-37 reduced production of Th2 cytokines in allergen-activated MNCs from wild-type but not from IL-1R1–deficient mice and inhibited IL-33–induced Th2 cytokine release. Furthermore, rhIL-37 attenuated IL-1β– and IL-33–induced pro-inflammatory mediator expression in murine AEC cultures. In contrast to wild-type mice, hIL-37 had no effect on EAA in IL-1R1– or IL-33–deficient mice. We also observed that expression/production ratios of both IL-1β and IL-33 to IL-37 were dramatically increased in asthma patients compared to healthy controls.

Conclusion: IL-37 downregulates allergic airway inflammation by counterbalancing the disease-amplifying effects of IL-1β and IL-33.

KEYWORDS
asthma, asthma treatment, inflammation, interleukin



GRAPHICAL ABSTRACT
IL-37 target cells include many cells involved in asthma pathogenesis such as AECs, DCs, ILC2s, and Th2 cells. IL-37 limits the pro-inflammatory and disease-amplifying effects of IL-1 and IL-33 on these target cells. Patients with asthma display increased expression/production ratios of both IL-1β and IL-33 to IL-37.

1 | INTRODUCTION

Asthma, one of the most common chronic diseases worldwide,[1] is highly heterogeneous, with a range of endotypes.[2,3] However, all endotypes are associated with chronic airways inflammation, with most patients experiencing symptoms.[2,4,5] Mostly, this inflammatory response is orchestrated by T helper (Th) 2 cells and their characteristic array of cytokines that overwhelm the counterbalancing properties of cells like Th1 and/or regulatory T cells (Tregs).[6-9] Nevertheless, the mechanisms leading to development of allergic inflammation (and thus of asthma) remain elusive. In an attempt to understand these mechanisms, the role of innate immune functions and the breakdown of the local immuno-homoeostasis are being studied. Airway epithelial cells (AECs) and antigen-presenting cells (APCs) produce factors that amplify or dampen acute inflammatory reactions: On the one hand, they augment allergic airway inflammation and thus formation of pathophysiological hallmarks of asthma by releasing pro-inflammatory cytokines such as interleukin (IL) 1β and IL-6, while epithelium-derived factors such as IL-33 promote Th2 and innate lymphoid tissue cell 2 (ILC2) differentiation that direct allergic immune responses via release of IL-4, IL-5, and IL-13.[10-13] On the other hand, they maintain local immuno-homeostasis and so prevent tissue damage by the actions of anti-inflammatory factors including IL-10 and IL-37, and an impairment of this immunoregulatory function could be a central factor in the pathogenesis of chronic inflammatory diseases such as asthma.[14,15]

Originally described as a fundamental inhibitor of innate immune functions,[16,17] the member of the IL-1β cytokine family, IL-37, has

been shown to impact adaptive immune responses, with production reduced in children with asthma.[18-20] Moreover, local administration of IL-37 not only reduces allergic airway inflammation but also ameliorates hallmarks of experimental allergic asthma (EAA) in mice. On a cellular basis, administration of IL-37 reduces the release of Th2 type cytokines and of several pro-inflammatory mediators, indicating that IL-37 impacts different cell types involved in asthma pathogenesis.[18,21-23] The anti-inflammatory properties of IL-37 are mediated by the IL-37 receptor complex composed of IL-1R5 and IL-1R8: Initial binding of monomeric IL-37 to IL-1R5 is followed by recruitment of IL-1R8, which acts as a co-receptor and transduces anti-inflammatory signals by suppressing NF-κB (nuclear factor κ-light-chain-enhancer of activated B cells) and MAPK (mitogen-activated protein kinase) and by activating the signaling molecules Mer, PTEN (Phosphatase and Tensin homolog), STAT (signal transducer and activator of transcription proteins) 3, and dok (downstream of kinase).[16,17,24,25] Since impaired production of IL-37 in patients with asthma could lead to dysregulation of allergic inflammation, clarification of the underlying mechanism potentially identifies new targets for therapeutic intervention. The aim of this study was therefore to elucidate the mechanism(s) by which IL-37 controls allergic inflammation. Our hypothesis is that IL-37 directly inhibits the pro-inflammatory effects of other members of the IL-1 family namely IL-1β and IL-33.

2 | MATERIALS AND METHODS

2.1 | Analysis of publicly available single-cell RNA-seq (scRNA-seq) data

Overview analysis of publicly available scRNA-seq data from healthy Human individuals[26,27] and control Mice[28,29] was performed using Scanpy (v.1.6.0.)[30] and Seurat (v.4.0.1),[31] respectively.

2.2 | Animals and experimental protocol

Female wild-type (WT) C57BL/6 mice (Charles River Laboratories, Sulzfeld, Germany), IL-1R1,[32] and IL-33 deficient,[33] aged 6–8 weeks, were housed under specific pathogen-free conditions, receiving an ovalbumin (OVA)-free diet and water *ad libitum*. All experimental procedures were approved by the animal ethics committee at the MELUND, Kiel, Germany (V312-72241.123–3(83-7/11), V244-28793/2016(44-4/16), and V244-6919/2018(19-3/18)). Induction of EAA and administration of IL-37 were performed as described previously.[18] For details, see the Supporting information.

Bronchoalveolar lavage (BAL) and differential cell count were performed as previously described.[34] Lung specimens were sampled and prepared for immunohistochemistry and quantitative morphology as previously described,[35] as were immunohistochemistry and quantitative morphology,[36] isolation and stimulation of mononuclear cells (MNCs) and $CD4^+$ T cells in cell culture,[36] and assessment of cytokine levels.[36] Furthermore, laser-capture microdissection,[37] whole genome array analysis of RNA samples,[38] air-liquid interface (ALI) culture of murine tracheal epithelial cells,[36] and reverse transcription and quantitative real-time polymerase chain reaction on murine and human samples were all performed as previously described.[37,39] For details, see online supplement.

2.3 | Cross-sectional human study cohort and protocol

Patient characteristics can be found in Table S1. Patients with asthma discontinued corticosteroid medication (inhaled and oral) for two weeks before analysis. For this analysis, we examined twelve patients with grass-pollen allergic asthma with hay fever, and 23 age-matched healthy controls during pollen season. All patients reported clinical symptoms to grass pollen, however, none of the control subjects. Clinical outcomes were assessed using the GINA (Global Initiative for Asthma) score,[40] striving for an optimal individual therapy setting. The study was approved by the ethics commission of the Technical University of Munich (5534/12). After written and informed consent, and in accordance with the Helsinki Declaration, induced sputum and nasal secretions were obtained from patients during the grass-pollen season from May to July 2014 and 2015 and analyzed as previously described.[41-43]

2.4 | Statistics

If not stated otherwise, results are presented as mean ± SEM. One-way analysis of variance with subsequent Tukey`s test was used to determine significance of differences. Human samples from each group were summarized taking the mean ± SD. Mann–Whitney tests were used to determine significant differences in expression ratios. Statistically significant differences were defined as p values $^*p < .05$, $^{**}p < .01$, $^{***}p < .001$, and $^{****}p < .0001$.

3 | RESULTS

3.1 | IL-1R5 and IL-1R8 are expressed by various cells associated with asthma pathogenesis

We have previously shown that topic treatment with rhIL-37 reduces the pathophysiologic features of EAA, including mucus hyperproduction, allergic airway inflammation, and release of Th2 type cytokines.[25] In order to determine which cells are influenced by IL-37, and to subsequently reveal its mechanism of action on a cellular level, we first analyzed the cellular expression of the two chains forming the IL-37 receptor signaling complex, namely IL-1R5 and IL-1R8. In human cells, we found mRNA expression of both receptor chains in a variety of cells associated with asthma pathogenesis, including $CD4^+$ T cells, dendritic cells (DCs), ILCs (Suppl. Figure 1A,B), and different AECs (Figure 1A). Despite differences in the expression level,

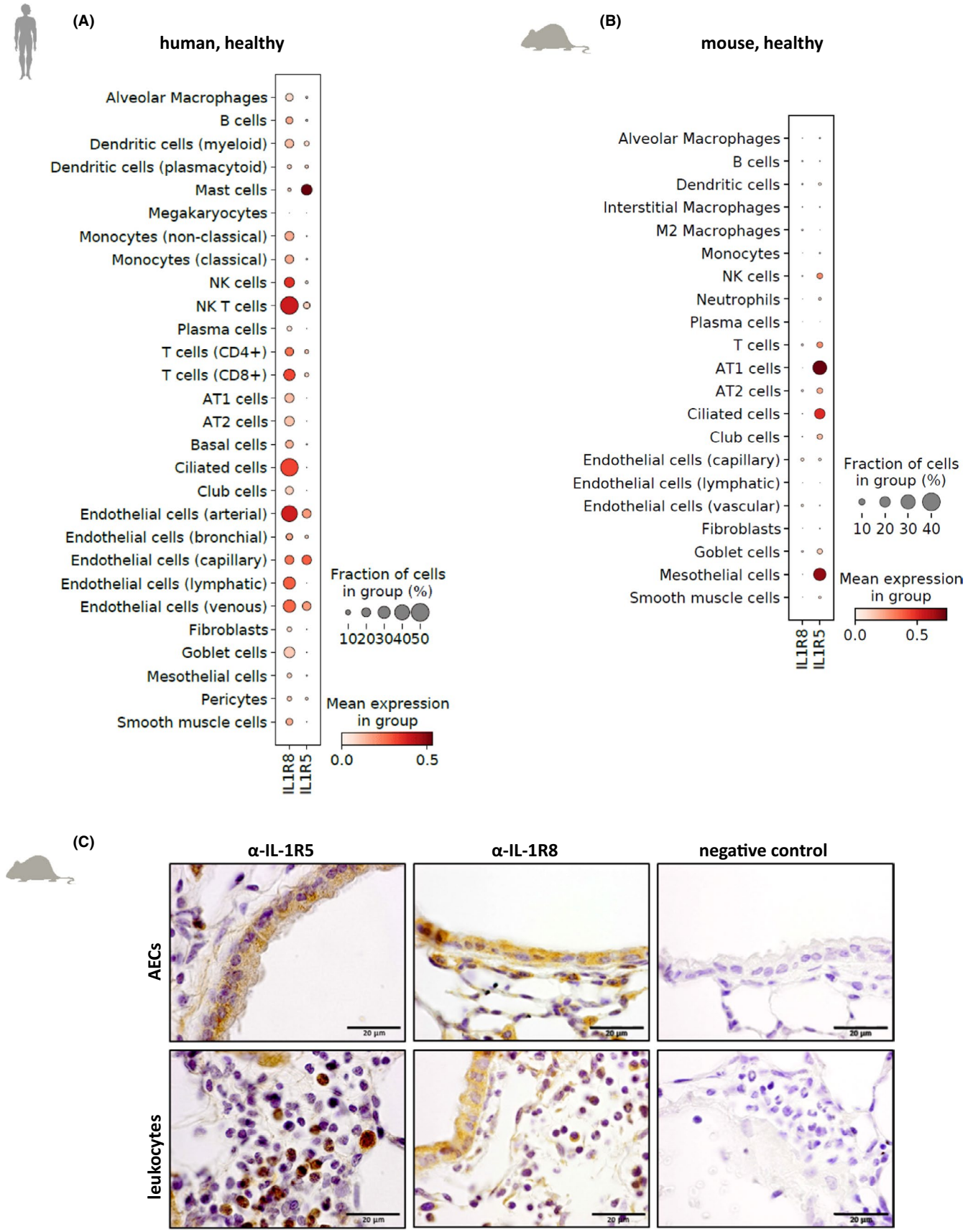

(A)
human, healthy
Alveolar Macrophages
B cells
Dendritic cells (myeloid)
Dendritic cells (plasmacytoid)
Mast cells
Megakaryocytes
Monocytes (non-classical)
Monocytes (classical)
NK cells
NK T cells
Plasma cells
T cells (CD4+)
T cells (CD8+)
AT1 cells
AT2 cells
Basal cells
Ciliated cells
Club cells
Endothelial cells (arterial)
Endothelial cells (bronchial)
Endothelial cells (capillary)
Endothelial cells (lymphatic)
Endothelial cells (venous)
Fibroblasts
Goblet cells
Mesothelial cells
Pericytes
Smooth muscle cells
IL1R8
IL1R5
Fraction of cells in group (%)
10 20 30 40 50
Mean expression in group
0.0 0.5
(B)
mouse, healthy
Alveolar Macrophages
B cells
Dendritic cells
Interstitial Macrophages
M2 Macrophages
Monocytes
NK cells
Neutrophils
Plasma cells
T cells
AT1 cells
AT2 cells
Ciliated cells
Club cells
Endothelial cells (capillary)
Endothelial cells (lymphatic)
Endothelial cells (vascular)
Fibroblasts
Goblet cells
Mesothelial cells
Smooth muscle cells
IL1R8
IL1R5
Fraction of cells in group (%)
10 20 30 40
Mean expression in group
0.0 0.5
(C)
α-IL-1R5
α-IL-1R8
negative control
AECs
leukocytes
20 µm

FIGURE 1 IL-1R5 and IL-1R8 are expressed by various cells associated with asthma pathogenesis. (A) Gene expression of IL-1R5 and IL-1R8 in different cell types in the human lung assessed by single-cell RNA-seq. (B) Gene expression of IL-1R5 and IL-1R8 in different cell types in the murine lung assessed by single-cell RNA-seq. (C) Immunohistochemically stained lung cross-sections of EAA mice with α-IL-1R5 and α-IL-1R8 antibodies. An isotype-matched IgG was used as negative control instead of the primary antibody. Scale bar, 20 μm. AEC: airway epithelial cells, AT1: alveolar epithelial type 1 cells, AT2: alveolar epithelial type 2 cells, IL-1R5: interleukin-1 receptor 5, IL-1R8: interleukin-1 receptor 8, and ILC: innate lymphoid tissue cells

we further confirmed mRNA levels of both chains for T cells, DCs, ILCs, and AECs (Figure 1B) in mice and on protein level for AECs and inflammatory cells infiltrating airway tissue under the pathologic conditions of EAA (Figure 1C), suggesting that these cells potentially respond to IL-37.

3.2 | IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs but not in anti-CD3/CD28 restimulated Th2 cells: role of IL-10

In mouse models of inflammatory diseases, such as contact hypersensitivity and atherosclerosis, the inhibitory effects of IL-37 are associated with recruitment of Tregs and tolerogenic DCs that downregulate inflammation by release of anti-inflammatory IL-10.[44-46] Therefore, we determined the levels of IL-10 in BALF of mice with EAA. Remarkably, IL-37 treatment did not enhance IL-10 levels in BALF. Accordingly, addition of IL-37 did not increase IL-10 release in OVA-restimulated MNCs from these animals (Figure 2A,B) indicating that IL-37 does not control allergic inflammation through induction of IL-10 release in Tregs or DCs. Thus, we investigated the effect of IL-37 on Th2 cell stimulation, since these cells are pivotal for orchestrating allergic inflammation. CD4+ T cells isolated from OVA-sensitized animals were restimulated in vitro by anti-CD3/CD28, which resulted in markedly increased production of the Th2 type cytokines IL-4, IL-5, and IL-13. The addition of IL-37 had no significant effect on these cytokine levels (Figure 2C) indicating that IL-37 does not affect T-cell activation via TCR/CD80/CD86. We therefore studied the effect of IL-37 on an allergen-specific restimulation of Th2 cells by stimulating MNCs from OVA-sensitized animals that include DCs, ILCs, and OVA-specific Th2 cells with OVA.[47] This led to enhanced production of IL-4, IL-5, and IL-13 (Figure 2D); IL-37 significantly diminished the release of these cytokines mirroring the effects of IL-37 treatment in animals with EAA. IL-37 induced no effects on either restimulated CD4+ T cells or MNCs.

3.3 | Expression of IL-1β– and IL-33–pathway members is upregulated in EAA and differentially regulated by IL-37 treatment

To get a further insight into the mechanisms through which IL-37 downregulates allergen-triggered airway inflammation, we compiled gene expression profiles of airway tissues from healthy mice, mice with EAA, and mice with EAA treated with IL-37. As expected, in animals with EAA numerous genes associated with inflammatory pathways were upregulated compared to healthy controls, including those triggered by IL-1β and IL-33 (Suppl. Figure 3A,C, Tables S2, Table S4), two pro-inflammatory cytokines associated with the pathogenesis of asthma.[48-51] Importantly, these two pathways were differentially expressed after IL-37 treatment, with several target genes downregulated (Suppl. Figure 3B,D, Tables S3, Table S5).

3.4 | IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs dependent on IL-1β and IL-33 signaling

Based on the finding that increased production of IL-1β in OVA-restimulated MNCs (Figure S4) is not observed in anti-CD3/CD28 restimulated $CD4^+$ cells, we hypothesized that IL-37 may exert its regulatory functions on MNCs by interfering with pro-inflammatory IL-1β and/or IL-33 signals that augment cytokine release of both, Th2 cells and ILC2s.[11-13] We therefore repeated OVA-restimulation of MNCs from OVA-sensitized IL-1R1–deficient mice that cannot react to IL-1β. Again, OVA-restimulation resulted in increased production of IL-4, IL-5, and IL-13, although to a lesser extent than MNCs from WT animals. However, in contrast to WT cells, co-administration of IL-37 did not result in any reduction in Th2 type cytokines (Figure 3A). The same effect was observed in IL-37–treated WT cells, when IL-1-signaling was inhibited by IL-1Ra: As observed in MNCs from IL-1R1–deficient mice, IL-1Ra administration lowered Th2 type cytokine release. Consistent with the experiments with IL-1R1–deficient cells, co-administration of IL-37– to IL-1Ra–treated and OVA-stimulated MNCs did not show any further reduction in Th2 type cytokine release (Figure 3B).

In contrast to IL-1β, which is produced by APCs, IL-33 is not produced in MNC cultures. However, since IL-33 exerts considerable effects on Th2 cells and ILCs,[11-13,52,53] we further examined whether IL-37 could also impact this cytokine. Therefore, we added IL-33 to allergen-restimulated MNCs from OVA-sensitized WT animals. We observed enhanced production of IL-5 and IL-13, but not IL-4; not unexpectedly, production of IL-5 and IL-13 was largely prevented by co-administration of IL-37 (Figure 3C).

3.5 | IL-37 attenuates IL-1β– and IL-33–induced expression of pro-inflammatory mediators in AECs

Similar to Th2 cells, AECs not only play a significant role in the pathogenesis of asthma but also are putative target cells for IL-37 (Figure 1). Thus, we investigated the in vitro effect of IL-37 on IL-1β or IL-33 stimulation by culturing murine AECs under

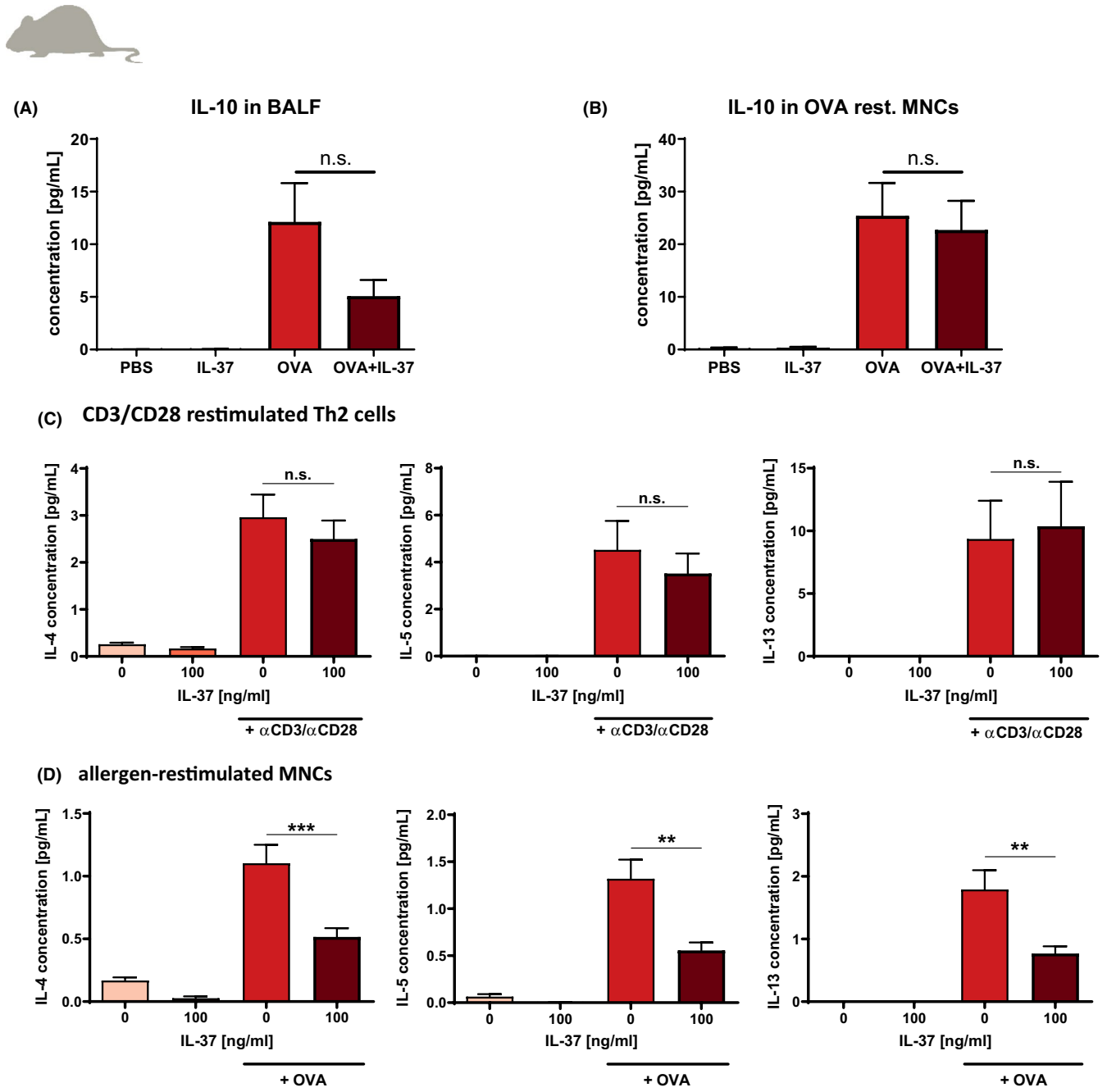


FIGURE 2 IL-37 attenuates Th2 type cytokine production in allergen-restimulated MNCs but not in anti-CD3/CD28 restimulated Th2 cells: role of IL-10. (A) Concentration of IL-10 in BALF. (B) MNCs were isolated from wild-type mice sensitized to OVA and restimulated with 50 μg/ml OVA for 48 h. (C) $CD4^+$ T lymphocytes were isolated from mice sensitized to OVA and restimulated with α-CD3/α-CD28 coated beads in the presence or absence of 100 ng/ml rhIL-37 for 48 h. (D) MNCs were isolated from mice sensitized to OVA and restimulated with OVA in the presence or absence of 100 ng/ml rhIL-37 for 48 h. Cytokine concentrations in BALF and cell culture supernatants were assessed by cytometric bead array. Cytokine concentrations are shown as mean ± SEM. $N = 8$ (A, B) or 11 (C, D) biological repeats. Statistical significance was assessed using one-way analysis of variance and Tukey´s multiple comparison post hoc analyses, $^{**}p < .01$; $^{***}p < .001$. BALF: bronchoalveolar lavage fluid, MNC: mononuclear cells, OVA: ovalbumin, and PBS: phosphate buffer solution

ALI-conditions, before stimulation with either IL-1β or IL-33 in the presence or absence of IL-37. First, we examined the effect of IL-1β stimulation. We observed enhanced expression of pro-inflammatory molecules including cytokines such as *Il6* and *Tnf*, chemokines such as *Kc* and *Mcp1*, *Gmcsf*, as well as the adhesion molecule *Icam1*; each of these has been implicated in the development and perpetuation of allergic asthma.[54] Co-administration of recombinant IL-37 markedly reduced the level of mRNA coding for these genes on AECs (Figure 4A).

Stimulation of AECs with IL-33 also resulted in upregulation of *Gmcsf*, *Icam1*, and *Kc*, and importantly, upregulation of these pro-inflammatory molecules was also counterbalanced by co-administration of IL-37

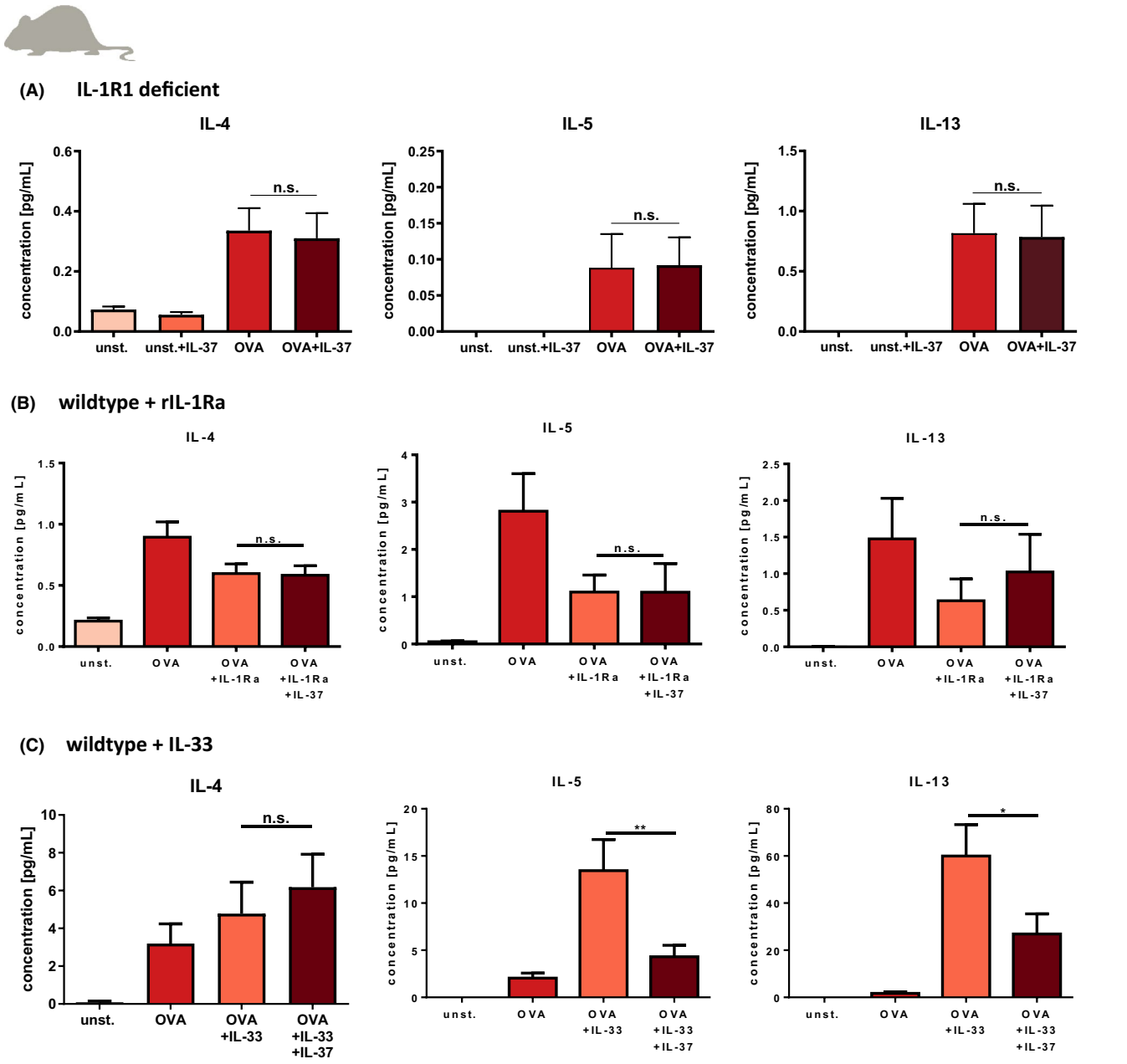


FIGURE 3 IL-37 attenuates Th2 cytokines production in allergen-restimulated MNCs dependent on IL-1β and IL-33 signaling. MNC were isolated from IL-1R1–deficient mice (A) or wild-type mice (B, C) sensitized to OVA and restimulated with 50 μg/ml OVA in the presence or absence of 100 ng/ml rhIL-37 and/or 1 μg/ml IL-1Ra (B) or 10 ng/ml IL-33 (C) for 48 h. Cytokine concentrations in the supernatant were assessed by cytometric bead array. Cytokine concentrations are shown as mean ± SEM. $N = 7$ (A), $N = 16$ (B), $N = 6$ (C) biological repeats. Statistical significance was assessed using one-way analysis of variance and Tukey´s multiple comparison post hoc analyses, $^{*}p < .05$; $^{**}p < .01$. IL-1Ra: interleukin-1 receptor antagonist, MNC: mononuclear cells, OVA: ovalbumin

(Figure 4B). IL-37 alone did not affect the expression of the respective genes in AECs.

3.6 | IL-37 downregulates allergic airway inflammation only in animals with a functional IL-1β and IL-33 signaling

Next, we investigated the effect of IL-37 on IL-1β and IL-33 under physiological conditions. WT, IL-1R1–, and IL-33–deficient mice with EAA were treated with IL-37. In WT animals, this resulted in reduced features of the disease, including eosinophil counts in BAL (Figure 5A), goblet cell (GC) hyperplasia (Figure 5B), and BAL levels of IL-4 (Figure 5C) and IL-6 (Figure 5D). IL-37 treatment did not reduce these features in IL-1R1–deficient animals (Figure 5A–D). In contrast, in IL-33–deficient animals, IL-37 treatment reduced BAL levels of IL-4 and IL-6, although not reaching statistical significance (Figure 5C,D). Eosinophil counts in BAL and GC hyperplasia remained largely unaffected (Figure 5A,B).

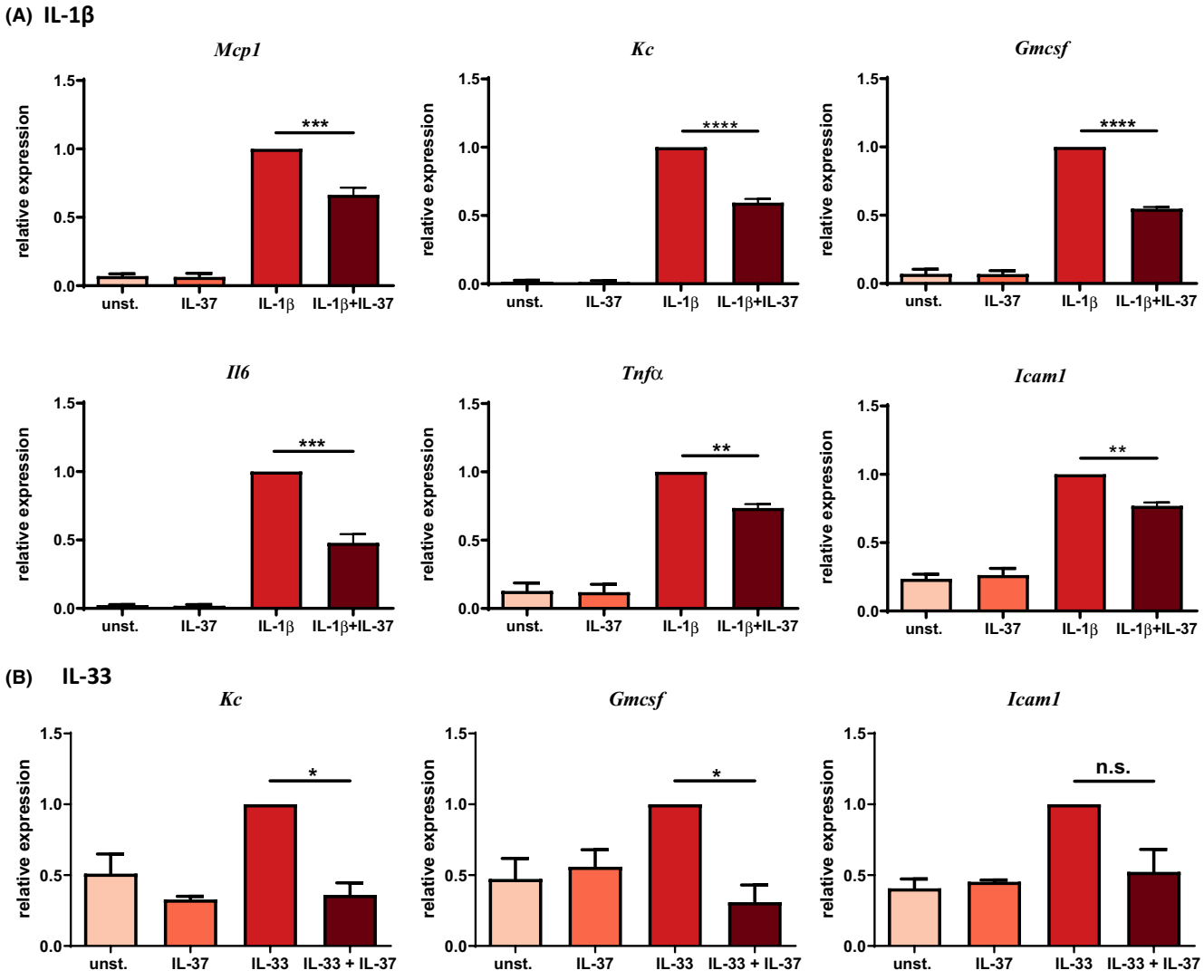


FIGURE 4 IL-37 attenuates IL-1β– and IL-33–induced expression of pro-inflammatory mediators in AECs. After differentiation at ALI for 8–11 days, cells were incubated with or without 100 ng/ml rhIL-37 and two hours later stimulated with 10 ng IL-1β (A) or IL-33 (B), respectively. Four hours later, mRNA was isolated and the expression of different genes was analyzed via qRT-PCR. *Rpl32* was used as housekeeping gene. Expression values were normalized to IL-1β alone group (A) or IL-33 alone group (B). The fold changes were calculated and are graphed as mean ± SEM of $N = 3$ biological repeats. Statistical significance was assessed using repeated measure one-way analysis of variance and Tukey´s multiple comparison post hoc analyses, $^{*}p < .05$; $^{**}p < .01$, $^{***}p < .001$; $^{****}p < .0001$. AEC: airway epithelial cells, ALI: air-liquid interface, *Gmcsf*: granulocyte-macrophage colony-stimulating factor gene, *Icam1*: intercellular adhesion molecule 1 gene, *Il6*: interleukine-6 gene, *KC*: keratinocytes-derived chemokine gene, *Mcp1*: monocyte chemotactic protein-1 gene, qRT-PCR: quantitative real-time polymerase chain reaction, and *Tnfa*: tumor necrosis factor α gene

3.7 | Lower and upper airways in asthma display increased expression/production ratios of IL-1β/IL-37 and IL-33/IL-37

Since our in vitro and in vivo experiments demonstrated that IL-37 limits the pro-inflammatory effects of IL-1β and IL-33 of cells in the pathogenesis of asthma (eg, MNCs and AECs), we hypothesized that patients with asthma exhibit an imbalance between anti-inflammatory IL-37 versus pro-inflammatory IL-1β and IL-33. Since glucocorticoids directly and indirectly inhibit the expression and production of IL-1β,[55-57] IL-33,[58-60] and IL-37,[61] we determined the mRNA expression and secreted protein levels of these cytokines in sputum cells of the lower airways and nasal secretions from healthy individuals and patients with allergic asthma (after glucocorticoid therapy had been discontinued) (Figure 6 and Suppl. Table S1). Asthma patients showed significantly increased expression levels for *Il1beta*, *Il33*, but decreased *Il37* mRNA expression

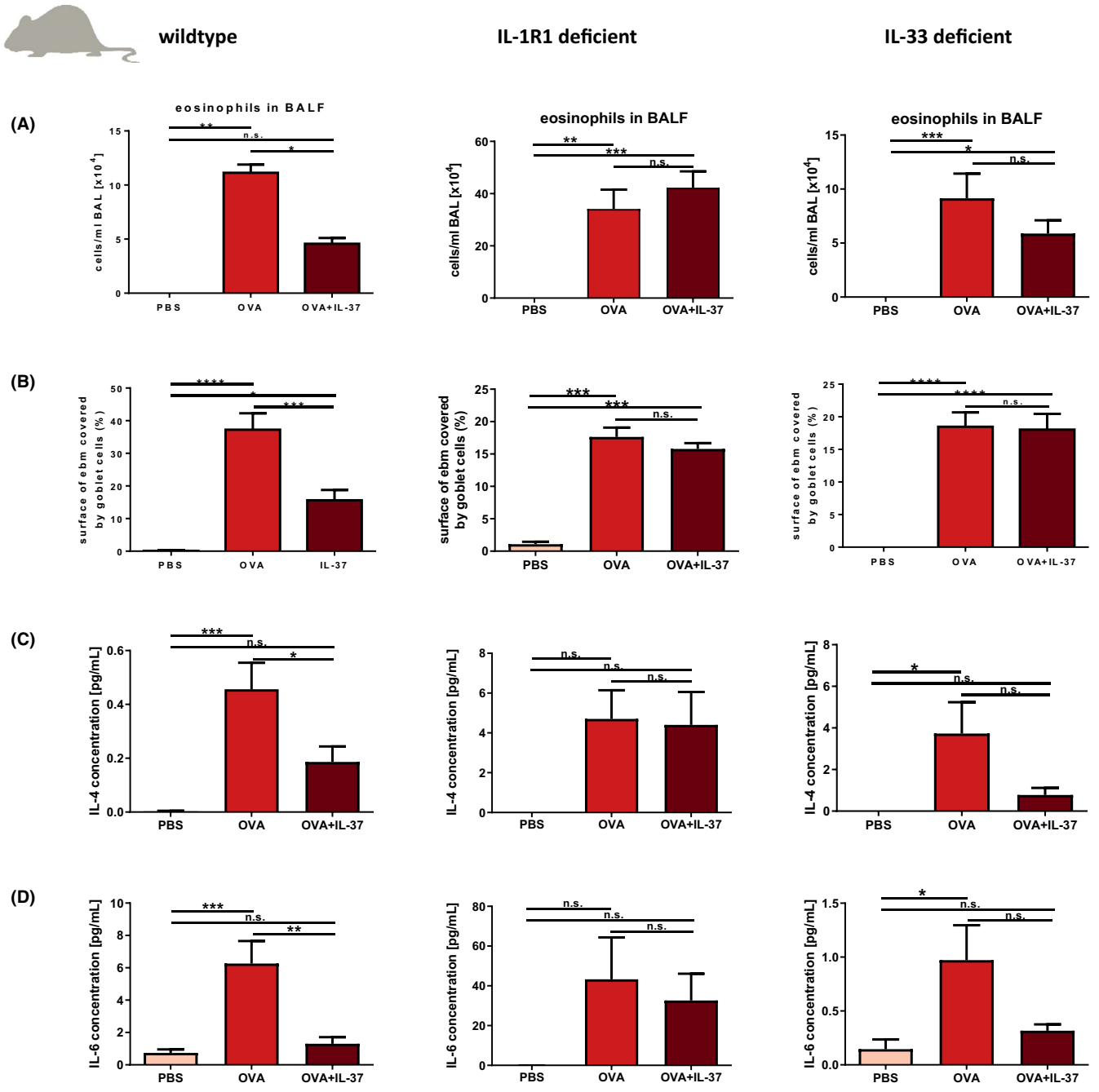


FIGURE 5 IL-37 downregulates allergic airway inflammation only in animals with a functional IL-1β and IL-33 signaling. (A) Total numbers of eosinophils in BALF, (B) area of EBM covered by goblet cells per epithelial basal membrane, and (C, D) cytokine level in BALF of healthy (PBS), asthmatic (OVA) and IL-37–treated asthmatic (OVA+IL-37) wild-type, IL-1R1–deficient, and IL-33–deficient mice. Data are shown as mean ± SEM. $N = 6–9$. Statistical significance was assessed using ordinary one-way analysis of variance and Tukey´s multiple comparison post hoc analyses, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. BALF: bronchoalveolar lavage fluid, EBM, epithelial basal membrane, OVA: ovalbumin, and PBS: phosphate buffer solution

in sputum cells (Figure 6A–C). Accordingly, patients with asthma displayed significantly higher expression ratios of *Il1beta* and *Il33* to *Il37* (Figure 6D,E). These differences were also confirmed at the protein level in sputum supernatants (Figure 6F–J) and nasal lining fluids (Figure 6K–O), suggesting an impaired counterbalance of these pro-inflammatory cytokines by IL-37.

4 | DISCUSSION

This study aimed to elucidate the mechanisms through which IL-37 controls allergic airway inflammation underlying the development of asthma. We identified various cells involved in the pathogenesis of asthma (including Th2 cells, DCs, ILC2s, and AECs) as potential responders to this cytokine and demonstrated

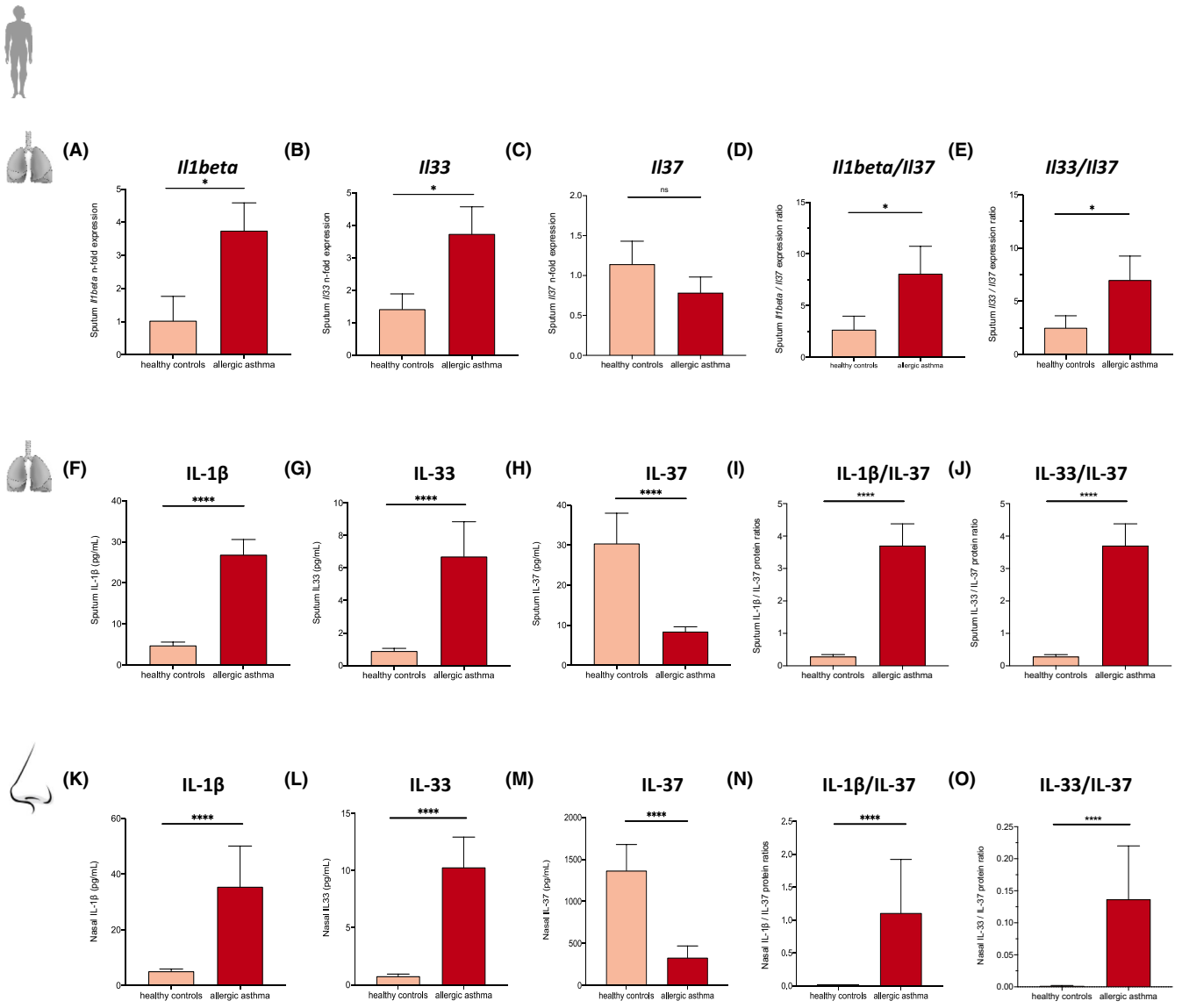


FIGURE 6 Lower and upper airways in asthma display increased expression/production ratios of IL-1β/IL-37 and IL-33/IL-37. Expression levels of (A) *Il1beta*, (B) *Il33*, and (C) *Il37* as well as ratio of expression levels of (D) *Il1beta* to *Il37* or (E) *Il33* to *Il37* in induced sputum cells from healthy controls and patients with allergic asthma. Secreted protein levels were measured in sputum supernatants of healthy controls and patients suffering from allergic asthma for (F) IL-1β, (G) IL-33, (H) IL-37 and the ratio of secreted levels of (I) IL-1β to IL-37 and (J) IL-33 to IL-37. Levels of secreted cytokines were also measured in upper airway lining fluids in healthy controls ($n = 23$) and patients suffering from allergic asthma ($n = 12$) for (K) IL-1β, (L) IL-33, (M) IL-37 and the ratio of secreted levels of (N) IL-1β to IL-37 and (O) IL-33 to IL-37. Data are presented as mean ± SD. Statistical significance was assessed using Mann–Whitney tests, $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$; $^{****}p < .0001$

that IL-37 limits the pro-inflammatory signals of IL-1β and IL-33 on a cellular level as well as in an EAA mouse model. Our additional finding that adult patients with asthma had enhanced expression/production ratios of the pro-inflammatory IL-1β and IL-33 to its anti-inflammatory counterpart IL-37 further supports the concept that impaired IL-37 production and, thus an impaired capacity to counterbalance pro-inflammatory signals in order to maintain the local immuno-homoeostasis, is of central importance in asthma pathogenesis.

We have previously shown that the therapeutic effects of local IL-37 administration require IL-1R5 and IL-1R8,[25] acting as the receptor for this cytokine.[17] Expression of these receptor chains was confirmed in a variety of cells involved in asthma pathogenesis in both, humans and mice. The differences in the expression level of the respective receptor chains between both species could be explained by the lack of pro-inflammatory stimuli that impact the expression of IL-1R5 and IL-1R8 in mice housed under specific pathogen-free conditions.[62] We hypothesized that co-expression of both receptors indicates putative responders to IL-37 and selected MNCs (including Th2 cells, ILC2s, and DCs) and AECs for further experiments, since they play central roles in asthma pathogenesis: Th2 cells and ILC2s orchestrate allergic inflammatory reactions and

enhance mucus production by releasing the Th2 type cytokines IL-4, IL-5, and IL-13.[63-65] In turn, AECs provide part of the physical barrier of the airways that is repeatedly exposed to allergens and pathogens, which trigger the release of several pro-inflammatory mediators (eg, TNF-α, IL-6, and IL-8/KC) thereby initializing and supporting acute inflammatory reactions.[66]

Since successful treatment of EAA in mice is associated with a reduced inflammatory response in the airways, we first checked for an enhanced IL-10 release (eg, by Tregs or DCs) as described in other mouse models of inflammatory disease.[44,45] As we found no increased release of IL-10 in vivo or in vitro, such an effect can be excluded. This rather surprising finding suggests that the short-term effects of IL-37 as described in this study are different from its long-term effects caused by ten weeks of treatment or transgenic overexpression.[18] We therefore further tested whether IL-37 impacts Th2 cell restimulation with anti-CD3/CD28. Unexpectedly, this was not the case, indicating that IL-37 does not interfere directly with the activation of these cells via the TCR and/or co-stimulation via CD80/86. Subsequently, we mimicked allergen-specific restimulation of MNCs isolated from OVA-sensitized animals with its specific antigen. Co-culture with IL-37 resulted in significant reduction in Th2 type cytokine production, consistent with our previous animal experiments. In contrast to our first experiment, MNC cultures comprised Th2 cells, ILC2s, and APCs, in which IL-37 could affect activation of APCs and ILC2s or interfere with the signals provided by these cells.[16] Thus, we returned to the animal model and created differential gene expression profiles from airway tissues from mice with EAA undergoing IL-37 or sham treatment. Among the several pathways that revealed differential expression upon IL-37 treatment, we observed those induced by IL-1β and IL-33. Thus, we hypothesized that IL-37 could exert its anti-inflammatory effects by interfering with their signals. We started by delineating the interrelationship of IL-1β and IL-37, since IL-1β release was elevated in mice with EAA as well as in allergen-restimulated MNCs. IL-1β is central for the activation, development, and proliferation of Th2 cells and ILC2s.[11-13,67-73] Accordingly, increased release of IL-1β is observed in patients with asthma.[20,74,75] Furthermore, administration of IL-1β worsens allergic airway inflammation and hallmarks of EAA in mice,[76] while blocking of IL-1β signaling leads to the opposite.[77,78] Our in vitro findings are consistent since allergen-restimulation of MNCs from IL-1R1–deficient mice that cannot respond to IL-1β displayed markedly reduced release of IL-4, IL-5, and IL-13. This is also the case for OVA-restimulated MNCs from WT animals receiving IL-1Ra. Importantly, in both settings co-application of IL-37 had no further impact on the production of Th2 type cytokines. As the regulatory effect of IL-37 is not observed if the pro-inflammatory effect of IL-1β is absent, we suggest that IL-37 counterbalances the pro-inflammatory effect of IL-1β on MNCs. This is supported by both, the in vivo experiment demonstrating that IL-37 has no significant reduction on Th2 type cytokine production, airway eosinophilia, or mucus hyperproduction in IL-1R1–deficient mice, and also by the AEC ALI-cultures. Here, upregulation of pro-inflammatory molecules, which have been implicated in asthma pathogenesis and mouse models of EAA,[79,80] by IL-1β could be directly reduced by addition of IL-37. Hence, IL-37 limits the effects of IL-1β, in terms of both its promotion of Th2 type cytokine production by MNCs and the expression of pro-inflammatory molecules by AECs, ultimately suppressing its amplifying effects on allergic airway inflammation.

Since we also found IL-33 pathway members to be differentially expressed by IL-37 treatment of mice with EAA, we speculated on a counterbalancing effect of IL-37 on this pathway. Alike IL-1, IL-33 considerably impacts allergic inflammation: It promotes Th2 cell differentiation,[48,81] activates ILCs and enhances their production of IL-5 and IL-13,[11-13,49,50] and activates AECs and amplifies their release of pro-inflammatory mediators such as IL-8/KC.[82] Accordingly, local application of IL-33 alone to mice induces all the pathophysiologic features of EAA.[83] We therefore cultured allergen-restimulated MNCs with IL-33, which indeed resulted in markedly enhanced release of IL-5 and IL-13. Similarly, in AEC cultures, IL-33 stimulation resulted in upregulation of pro-inflammatory molecules including *gmcsf*, *icam1*, and *kc*. Strikingly, both effects were dampened by presence of IL-37. Furthermore, the effects of IL-37 on Th2 type cytokine levels in BAL, airway eosinophilia, and mucus production were considerably diminished in IL-33–deficient animals indicating an inhibiting effect of IL-37 on pro-inflammatory IL-33.

Finally, we sought to translate our experimental findings to the clinical situation. We determined the levels of IL-1β and IL-33 and their counterpart IL-37 in patients with mild allergic asthma who discontinued glucocorticoid treatment. Without the inhibiting effect of glucocorticoids on the release of all these cytokines,[55-61] patients with asthma had a dramatically enhanced ratio of both IL-1β to IL-37 and IL-33 to IL-37 as compared to healthy individuals. This predominant release of pro-inflammatory cytokines that is not counterbalanced by comparable IL-37 could be the basis for the persistence of allergic airway inflammation in adult patients. The reasons for this imbalance and for an impaired release of IL-37 in patients with asthma patients remain unclear. However, this finding is in line with a previous study that described loss of anti-inflammatory functions of four different genetic variants of IL-37 in patients with gout, characterized by severe IL-1-mediated joint inflammation.[84] Together with this, our findings highlight the therapeutic potential of recombinant IL-37 for the treatment of inflammatory diseases.

Taken together, we demonstrate that IL-37 exerts its anti-inflammatory action on allergic airway inflammation by counterbalancing the disease-amplifying effects of two pro-inflammatory cytokines that are central to asthma pathogenesis, namely IL-1β and IL-33. We demonstrated this mechanism in MNCs and AECs, which play pivotal roles in asthma pathogenesis. However, we suggest that the same mechanism also affects other cells that are influenced by IL-1β and/or IL-33 and IL-37. As we did not find any signs for the induction of IL-10, we suggest that our findings describe a new mechanism by which IL-37 directly controls allergic inflammation. We suggest that over prolonged time this mechanism could contribute to the development of tolerogenic DCs and/or Tregs by persistent abrogation of pro-inflammatory signals, which is in line with the recent presentation of IL-37 as an active inhibitor of trained immunity

effects.[85] Together with its previously described balancing effects on the immunometabolism, this regulatory effect of IL-37 on allergic inflammation establishes IL-37 as a central factor for the maintenance of the local immuno-homoeostasis.[86,87] Consequently, these findings suggest specifically targeting these pro-inflammatory cytokine effects (eg, by compensating for the impaired IL-37 response or by inhibiting IL-1R3)[51] will be superior to glucocorticoid treatment that also decreases the inherent regulatory effects of IL-37.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. To T, Stanojevic S, Moores G, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. *BMC Public Health*. 2012;12:204.
2. Lötvall J, Akdis CA, Bacharier LB, et al. Asthma endotypes: a new approach to classification of disease entities within the asthma syndrome. *J Allergy Clin Immunol*. 2011;127(2):355-360.
3. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. 2012;18(5):716-725.
4. Anderson GP. Endotyping asthma: new insights into key pathogenic mechanisms in a complex, heterogeneous disease. *Lancet*. 2008;372(9643):1107-1119.
5. Fahy JV. Type 2 inflammation in asthma–present in most, absent in many. *Nat Rev Immunol*. 2015;15(1):57-65.
6. Haldar P, Pavord ID, Shaw DE, et al. Cluster analysis and clinical asthma phenotypes. *Am J Respir Crit Care Med*. 2008;178(3):218-224.
7. Moore WC, Meyers DA, Wenzel SE, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med*. 2010;181(4):315-323.
8. Woodruff PG, Boushey HA, Dolganov GM, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. *Proc Natl Acad Sci USA*. 2007;104(40):15858-15863.
9. Woodruff PG, Modrek B, Choy DF, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med*. 2009;180(5):388-395.
10. Lambrecht BN, Hammad H. The airway epithelium in asthma. *Nat Med*. 2012;18(5):684-692.
11. Ohne Y, Silver JS, Thompson-Snipes LuAnn, et al. IL-1 is a critical regulator of group 2 innate lymphoid cell function and plasticity. *Nat Immunol*. 2016;17(6):646-655.
12. Frisbee AL, Saleh MM, Young MK, et al. IL-33 drives group 2 innate lymphoid cell-mediated protection during Clostridium difficile infection. *Nat Commun*. 2019;10(1):1-13.
13. Salmond RJ, Mirchandani AS, Besnard A-G, Bain CC, Thomson NC, Liew FY. IL-33 induces innate lymphoid cell–mediated airway inflammation by activating mammalian target of rapamycin. *J Allergy Clin Immunol*. 2012;130(5):1159-1166.
14. Wegmann M. Targeting cytokines in asthma therapy: could IL-37 be a solution? *Expert Rev Respir Med*. 2017;11(9):675-677.
15. Lunding L, Schröder A, Wegmann M. Allergic airway inflammation: unravelling the relationship between IL-37, IL-18Rα and Tir8/SIGIRR. *Expert Rev Respir Med*. 2015;9(6):739-750.
16. Nold MF, Nold-Petry CA, Zepp JA, Palmer BE, Bufler P, Dinarello CA. IL-37 is a fundamental inhibitor of innate immunity. *Nat Immunol*. 2010;11(11):1014-1022.
17. Nold-Petry CA, Lo CY, Rudloff I, et al. IL-37 requires the receptors IL-18Rα and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction. *Nat Immunol*. 2015;16(4):354-365.
18. Lunding L, Webering S, Vock C, et al. IL-37 requires IL-18Rα and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice. *Allergy*. 2015;70(4):366-373.
19. Raedler D, Ballenberger N, Klucker E, et al. Identification of novel immune phenotypes for allergic and nonallergic childhood asthma. *J Allergy Clin Immunol*. 2015;135(1):81-91.
20. Charrad R, Berraïes A, Hamdi B, Ammar J, Hamzaoui K, Hamzaoui A. Anti-inflammatory activity of IL-37 in asthmatic children: Correlation with inflammatory cytokines TNF-α, IL-β, IL-6 and IL-17A. *Immunobiology*. 2016;221(2):182-187.

21. Zhu J, Dong J, Ji LU, et al. Anti-allergic inflammatory activity of interleukin-37 is mediated by novel signaling cascades in human eosinophils. *Front Immunol.* 2018;9:1445.
22. Huang N, Liu K, Liu J, et al. Interleukin-37 alleviates airway inflammation and remodeling in asthma via inhibiting the activation of NF-κB and STAT3 signalings. *Int Immunopharmacol.* 2018;55:198-204.
23. Lv J, Xiong Y, Li W, et al. IL-37 inhibits IL-4/IL-13-induced CCL11 production and lung eosinophilia in murine allergic asthma. *Allergy.* 2018;73(8):1642-1652.
24. Cavalli G, Dinarello CA. Suppression of inflammation and acquired immunity by IL-37. *Immunol Rev.* 2018;281(1):179-190.
25. Lunding L, Webering S, Vock C, et al. IL-37 requires IL-18Rα and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice. *Allergy.* 2015;70(4):366-373.
26. Mayr CH, Simon LM, Leuschner G, et al. Integrated single cell analysis of human lung fibrosis resolves cellular origins of predictive protein signatures in body fluids. *medRxiv.* Published online January 27, 2020:2020.01.21.20018358. https://doi.org/10.1101/2020.01.21.20018358
27. Strunz M, Simon LM, Ansari M, et al. Alveolar regeneration through a Krt8+ transitional stem cell state that persists in human lung fibrosis. *Nat Commun.* 2020;11(1):1-20.
28. Wolf FA, Angerer P, Theis FJ. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol.* 2018;19(1):15.
29. Adams TS, Schupp JC, Poli S, et al. Single-cell RNA-seq reveals ectopic and aberrant lung-resident cell populations in idiopathic pulmonary fibrosis. *Sci. Adv.* 2020;6(28):eaba1983.
30. Ghaedi M, Shen ZY, Orangi M, et al. Single-cell analysis of RORα tracer mouse lung reveals ILC progenitors and effector ILC2 subsets. *J Exp Med.* 2020;217(3):jem.20182293
31. Stuart T, Butler A, Hoffman P, et al. Comprehensive Integration of Single-Cell Data. *Cell.* 2019;177(7):1888-1902.
32. Glaccum MB, Stocking KL, Charrier K, et al. Phenotypic and functional characterization of mice that lack the type I receptor for IL-1. *J Immunol.* 1997;159(7):3364-3371.
33. Hardman CS, Panova V, McKenzie ANJ. IL-33 citrine reporter mice reveal the temporal and spatial expression of IL-33 during allergic lung inflammation. *Eur J Immunol.* 2013;43(2):488-498.
34. Lunding LP, Webering S, Vock C, et al. Poly(inosinic-cytidylic) acid-triggered exacerbation of experimental asthma depends on IL-17A produced by NK cells. *J Immunol.* 2015;194(12):5615-5625.
35. Wegmann M, Lunding L, Orinska Z, Wong DM, Manz RA, Fehrenbach H. Long-term bortezomib treatment reduces allergen-specific IgE but fails to ameliorate chronic asthma in mice. *Int Arch Allergy Immunol.* 2012;158(1):43-53.
36. Webering S, Lunding LP, Vock C, et al. The alpha-melanocyte-stimulating hormone acts as a local immune homeostasis factor in experimental allergic asthma. *Clin Exp Allergy.* 2019;49(7):1026-1039.
37. Vock C, Yildirim AÖ, Wagner C, et al. Distal airways are protected from goblet cell metaplasia by diminished expression of IL-13 signalling components. *Clin Exp Allergy.* 2015;45(9):1447-1458.
38. Zissler UM, Jakwerth CA, Guerth FM, et al. Early IL-10 producing B-cells and coinciding Th/Tr17 shifts during three year grass-pollen AIT. *EBioMedicine.* 2018;36:475-488.
39. Zissler UM, Chaker AM, Effner R, et al. Interleukin-4 and interferon-γ orchestrate an epithelial polarization in the airways. *Mucosal Immunol.* 2016;9(4):917-926.
40. Reddel HK, Bateman ED, Becker A, et al. A summary of the new GINA strategy: a roadmap to asthma control. *Eur Respir J.* 2015;46(3):622-639.
41. Eckrich J, Zissler UM, Serve F, et al. Airway inflammation in mild cystic fibrosis. *J Cyst Fibros.* 2017;16(1):107-115.
42. Zissler UM, Ulrich M, Jakwerth CA, et al. Biomatrix for upper and lower airway biomarkers in patients with allergic asthma. *J Allergy Clin Immunol.* 2018;142(6):1980-1983.
43. Zissler UM, Jakwerth CA, Guerth F, et al. Allergen-specific immunotherapy induces the suppressive secretoglobin 1A1 in cells of the lower airways. *Allergy.* 2021;76(8):2461-2474.
44. Luo Y, Cai X, Liu S, et al. Suppression of antigen-specific adaptive immunity by IL-37 via induction of tolerogenic dendritic cells. *Proc Natl Acad Sci USA.* 2014;111(42):15178-15183.
45. Ji Q, Meng K, Yu K, et al. Exogenous interleukin 37 ameliorates atherosclerosis via inducing the Treg response in ApoE-deficient mice. *Sci Rep.* 2017;7(1):3310.
46. Osborne DG, Domenico J, Luo Y, et al. Interleukin-37 is highly expressed in regulatory T cells of melanoma patients and enhanced by melanoma cell secretome. *Mol Carcinog.* 2019;58(9):1670-1679.
47. Li Y, Chen S, Chi Y, et al. Kinetics of the accumulation of group 2 innate lymphoid cells in IL-33-induced and IL-25-induced murine models of asthma: a potential role for the chemokine CXCL16. *Cell Mol Immunol.* 2019;16(1):75-86.
48. Liew FY, Girard J-P, Turnquist HR. Interleukin-33 in health and disease. *Nat Rev Immunol.* 2016;16(11):676-689.
49. Kurowska-Stolarska M, Kewin P, Murphy G, et al. IL-33 induces antigen-specific IL-5+ T cells and promotes allergic-induced airway inflammation independent of IL-4. *J Immunol.* 2008;181(7):4780-4790.
50. Guo L, Wei G, Zhu J, et al. IL-1 family members and STAT activators induce cytokine production by Th2, Th17, and Th1 cells. *Proc Natl Acad Sci USA.* 2009;106(32):13463-13468.
51. Højen JF, Kristensen MLV, McKee AS, et al. IL-1R3 blockade broadly attenuates the functions of six members of the IL-1 family, revealing their contribution to models of disease. *Nat Immunol.* 2019;20(9):1138-1149.
52. Chu DK, Llop-Guevara A, Walker TD, et al. IL-33, but not thymic stromal lymphopoietin or IL-25, is central to mite and peanut allergic sensitization. *J Allergy Clin Immunol.* 2013;131(1):187-200.
53. Tjota MY, Hrusch CL, Blaine KM, Williams JW, Barrett NA, Sperling AI. Signaling through FcRγ-associated receptors on dendritic cells drives IL-33-dependent TH2-type responses. *J Allergy Clin Immunol.* 2014;134(3):706-713.
54. Lambrecht BN, Hammad H, Fahy JV. The cytokines of asthma. *Immunity.* 2019;50(4):975-991.
55. Amano Y, Lee SW, Allison AC. Inhibition by glucocorticoids of the formation of interleukin-1 alpha, interleukin-1 beta, and interleukin-6: mediation by decreased mRNA stability. *Mol Pharmacol.* 1993;43(2):176-182.
56. Levine SJ, Benfield T, Shelhamer JH. Corticosteroids induce intracellular interleukin-1 receptor antagonist type I expression by a human airway epithelial cell line. *Am J Respir Cell Mol Biol.* 1996;15(2):245-251.
57. Knudsen PJ, Dinarello CA, Strom TB. Glucocorticoids inhibit transcriptional and post-transcriptional expression of interleukin 1 in U937 cells. *J Immunol.* 1987;139(12):4129-4134.
58. Chan BCL, Lam CWK, Tam L-S, Wong CK. IL33: roles in allergic inflammation and therapeutic perspectives. *Front Immunol.* 2019;10:364.
59. Ayroldi E, Cannarile L, Migliorati G, Nocentini G, Delfino DV, Riccardi C. Mechanisms of the anti-inflammatory effects of glucocorticoids: genomic and nongenomic interference with MAPK signaling pathways. *FASEB J.* 2012;26(12):4805-4820.
60. Gorbacheva AM, Kuprash DV, Mitkin NA. Glucocorticoid Receptor Binding Inhibits an Intronic IL33 Enhancer and is Disrupted by rs4742170 (T) Allele Associated with Specific Wheezing Phenotype in Early Childhood. *Int J Mol Sci.* 2018;19(12):3956.
61. Song L, Qiu F, Fan Y, et al. Glucocorticoid regulates interleukin-37 in systemic lupus erythematosus. *J Clin Immunol.* 2013;33(1):111-117.

62. Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. *Immunol Rev.* 2018;281(1):8-27.
63. Stavnezer J. Immunoglobulin class switching. *Curr Opin Immunol.* 1996;8(2):199-205.
64. Nakayama T, Hirahara K, Onodera A, et al. Th2 cells in health and disease. *Annu Rev Immunol.* 2017;35:53-84.
65. Dougan M, Dranoff G, Dougan SK. GM-CSF, IL-3, and IL-5 family of cytokines: regulators of inflammation. *Immunity.* 2019;50(4):796-811.
66. Hiemstra PS, McCray PB, Bals R. The innate immune function of airway epithelial cells in inflammatory lung disease. *Eur Respir J.* 2015;45(4):1150-1162.
67. Manetti R, Barak V, Piccinni M-P, et al. Interleukin-1 favours the in vitro development of type 2 T helper (Th2) human T-cell clones. *Res Immunol.* 1994;145(2):93-100.
68. Kurt-Jones EA, Hamberg S, Ohara J, Paul WE, Abbas AK. Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. *J Exp Med.* 1987;166(6):1774-1787.
69. Greenbaum LA, Horowitz JB, Woods A, Pasqualini T, Reich EP, Bottomly K. Autocrine growth of $CD4^+$ T cells. Differential effects of IL-1 on helper and inflammatory T cells. *J Immunol.* 1988;140(5):1555-1560.
70. Berkman N, Robichaud A, Krishnan VL, et al. Expression of RANTES in human airway epithelial cells: effect of corticosteroids and interleukin-4, -10 and -13. *Immunology.* 1996;87(4):599-603.
71. Lilly CM, Nakamura H, Kesselman H, et al. Expression of eotaxin by human lung epithelial cells: induction by cytokines and inhibition by glucocorticoids. *J Clin Invest.* 1997;99(7):1767-1773.
72. Sukkar MB, Issa R, Xie S, Oltmanns U, Newton R, Chung KF. Fractalkine/CX3CL1 production by human airway smooth muscle cells: induction by IFN-gamma and TNF-alpha and regulation by TGF-beta and corticosteroids. *Am J Physiol Lung Cell Mol Physiol.* 2004;287(6):L1230-1240.
73. El-Shazly A, Berger P, Girodet P-O, et al. Fraktalkine produced by airway smooth muscle cells contributes to mast cell recruitment in asthma. *J Immunol.* 2006;176(3):1860-1868.
74. Tillie-leblond I, Pugin J, Marquette C-H, et al. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. *Am J Respir Crit Care Med.* 1999;159(2):487-494.
75. Borish L, Mascali JJ, Dishuck J, Beam WR, Martin RJ, Rosenwasser LJ. Detection of alveolar macrophage-derived IL-1 beta in asthma. Inhibition with corticosteroids. *J Immunol.* 1992;149(9):3078-3082.
76. Horiba M, Qutna N, Gendapodi P, et al. Effect of IL-1β and TNF-α vs IL-13 on bronchial hyperresponsiveness, β2-adrenergic responses and cellularity of bronchial alveolar lavage fluid. *Auton Autacoid Pharmacol.* 2011;31(3–4):37-49.
77. Wang C-C, Fu C-L, Yang Y-H, et al. Adenovirus expressing interleukin-1 receptor antagonist alleviates allergic airway inflammation in a murine model of asthma. *Gene Ther.* 2006;13(19):1414-1421.
78. Ritter M, Straubinger K, Schmidt S, et al. Functional relevance of NLRP3 inflammasome-mediated interleukin (IL)-1β during acute allergic airway inflammation. *Clin Exp Immunol.* 2014;178(2):212-223.
79. Fajt ML, Wenzel SE. Asthma phenotypes and the use of biologic medications in asthma and allergic disease: The next steps toward personalized care. *J Allergy Clin Immunol.* 2015;135(2):299-310.
80. Akdis M, Aab A, Altunbulakli C, et al. Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases. *J Allergy Clin Immunol.* 2016;138(4):984-1010.
81. Zhu J, Cote-Sierra J, Guo L, Paul WE. Stat5 activation plays a critical role in Th2 differentiation. *Immunity.* 2003;19(5):739-748.
82. Scott IC, Majithiya JB, Sanden C, et al. Interleukin-33 is activated by allergen- and necrosis-associated proteolytic activities to regulate its alarmin activity during epithelial damage. *Sci Rep.* 2018;8(1):3363.
83. Schmitz J, Owyang A, Oldham E, et al. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity.* 2005;23(5):479-490.
84. Klück V, van Deuren RC, Cavalli G, et al. Rare genetic variants in interleukin-37 link this anti-inflammatory cytokine to the pathogenesis and treatment of gout. *Ann Rheum Dis.* 2020;79(4):536-544.
85. Cavalli G, Tengesdal IW, Gresnigt M, et al. The anti-inflammatory cytokine interleukin-37 is an inhibitor of trained immunity. *Cell Rep.* 2021;35(1):108955.
86. Cavalli G, Justice JN, Boyle KE, et al. Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. *PNAS.* 2017;114(9):2313-2318.
87. Ballak DB, Li S, Cavalli G, et al. Interleukin-37 treatment of mice with metabolic syndrome improves insulin sensitivity and reduces pro-inflammatory cytokine production in adipose tissue. *J Biol Chem.* 2018;293(37):14224-14236.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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