IL-4Rα signalling in CD4+CD25+FoxP3+ T regulatory cells restrain airway inflammation via limiting local tissue IL-33

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**Graphical abstract**

![Graphical abstract showing immune cells and cytokines](https://jci.me/136206/pdf)
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Conflict of interest statement

The authors have declared that no conflict of interest exists.

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Abstract

Impaired tolerance to innocuous particles during allergic asthma has been linked to the increased plasticity of FoxP3+ regulatory T (Treg) cells, reprogramming into pathogenic effector cells, thus exacerbating airway disease. Failure in tolerance is suggested to be driven by TH2 inflammatory signals. The canonical IL-4Rα-signalling, an essential driver of TH2-type airway responses to allergens was investigated on its in vivo role on the regulatory function of FoxP3+ Tregs in allergic asthma. We used transgenic Foxp3creIL-4Rαlox and littermate control mice to investigate the role of IL-4/IL-13 signalling via T regs in a house dust mite (HDM)-induced allergic airway disease. We sensitised mice intratracheally on day 0 and challenged them on day 6-10 and analysed airway hyperresponsiveness (AHR), airway inflammation, mucus production and cellular profile on day 14. In the absence of IL-4Rα responsiveness on FoxP3+ Tregs, there was an exacerbated AHR and airway inflammation in HDM-sensitised mice. Interestingly, a reduced induction of FoxP3+ Tregs accompanied increased IL-33 “alarmin” production and innate lymphoid cells type 2 (ILC2) activation in the lung exacerbating airway hyperreactivity and lung eosinophilia. We conclude that IL-4Rα unresponsive FoxP3+ T regulatory cells results in exaggerated innate TH2-type, IL-33-dependent airway inflammation and a break in tolerance during allergic asthma.

Abbreviations

Foxhead box P3 (FoxP3), House dust mite (HDM), Airway hyperresponsiveness (AHR), Innate lymphoid cells type 2 (ILC2)
Introduction

Allergic asthma is a chronic dysregulated airway immune response to harmless ubiquitous environmental particles (1). Allergic asthma is characterized by aberrant T helper 2 (TH2) cells that drive the many hallmarks of the disease including airway hyperresponsiveness (AHR), goblet cell hyperplasia, eosinophilia, allergen-specific IgE, influx of effector TH2-associated cytokines (1). Allergic asthma is characterised by failed tolerogenic mechanisms to ubiquitous allergens, leading to uncontrolled TH2 airway responses (2–4). Tolerance to allergens is induced by fork head box P3 (FoxP3) CD25+ T regulatory (T reg) cells (5). Indeed, mice and humans with dysfunctional FOXP3 present with neonatal development of severe allergies characterized by hyper mucosal T\textsubscript{H}2 cells (6, 7).

T regulatory cells can induce tolerance in several ways and can target a broad array of cells including B and T cells, innate lymphoid cells (ILCs), antigen presenting cells (APCs) in diverse mechanisms that show complex specificity guided by local environmental cues and an inflammatory condition (8). These regulatory mechanisms include but not limited to suppression of pathogenic effector T cells through secretion of immunosuppressive anti-inflammatory IL-10 and TGF-\textbeta cytokines (4), consumption of IL-2 (9), cell-cell contact suppression with costimulatory receptors, cytotoxic T lymphocyte–associated protein 4 (CTLA) (10), inducible costimulator (ICOS) (11) and programmed death 1 (PD-1) (3), contact-dependent killing of APCs through perforin and granzyme B or depletion of peptide/major histocompatibility complex II from dendritic cells (12).

Tolerance to allergens at mucosal surfaces is induced by specialized Treg subset, called induced Tregs (iTregs), which are extrathymically derived and exert their immunosuppressive
function through IL-10 secretion (13). In allergic asthma, iTregs are generated de novo by specialized lung tissue resident macrophages through secretion of TGF-β and retinal dehydrogenases (14). TGF-β is important in the generation of antigen-specific FoxP3 Tregs and its abrogation in vivo leads to uncontrolled TH2 allergic asthma. (15). Although iTregs may be a major player in generation of tolerance to airborne allergens, other mechanisms of tolerance exist and include diversion of allergen-specific cells to non-TH2 cells, peripheral deletion of autoreactive cells to harmless allergens and allergen ignorance (16, 17). How TH2 cells break this tolerance and cause allergic asthma disease is not well understood.

TH2 cells can impair induction of Tregs either by hijacking them to become effector TH2 cells or by limiting their ability to act as active TH2 suppressor cells (15). These mechanisms include reprogramming of Tregs toward a pathogenic effector T cell phenotype leading to unrestrained TH2 airway inflammatory response (18–22). This paradigm of a TH2 dependent impairment of Treg function suggests a requirement for IL-4 and its receptor signalling on Tregs in allergic asthma. Mechanistically, recent studies have shown that a mutation in the interleukin 4 receptor alpha (IL-4Rα) in a position 709, where tyrosine is substituted for phenylalanine (IL-4RαF709) have increased STAT6 activity and exaggerated TH2 mucosal responses and food allergy (20). This mutation is in the tyrosine inhibitory motif and causes unrestrained STAT6 phosphorylation. This phenotype in murine models phenocopies that observed in human IL-4Rα with hyper-STAT6 activation (23). Another human polymorphism in IL-4Rα where glutamine is substituted for arginine at position 576 (IL-4RαQ576R) is associated with asthma exacerbations. Mice carrying a homozygous allele of Q576R show increased TH2 and TH17 airway responses to house dust mite (HDM) and impaired iTreg induction (24). These studies suggest that IL-4 and IL-4Rα signalling via downstream transcription factor STAT6, disrupt the differentiation of CD4+ T cells into FoxP3+
Tregs in vitro and also in vivo (25). Indeed, we and others have shown that deletion of the IL-4Rα in iTregs disrupts the Foxp3 stability promoting an increased TH2 immunity and clearance of *H. poly* (26) or susceptibility to *S. mansoni* (27).

All these studies point to mechanisms where IL-4 through its receptor, acts on FoxP3 iTregs inducing their differentiation and reprogramming them into pathogenic ex-FoxP3+ TH2 or ex-FoxP3+ TH17 (19, 24, 26, 28). Other studies have shown that overexpression of TH2-associated transcriptional factors such as Irf4 or Gata3 specifically on Tregs promotes their trans-differentiation into effector TH2 cells (29). Deletion of these transcriptional factors prunes the ability of iTregs to suppress TH2 immune responses (30). This highlights the role of a TH2 environment in impairing Treg tolerance in allergic disease. The functional role of IL-4Rα signalling on FoxP3+ Tregs during allergic disease is not completely clear. Here, we show that deletion of IL-4Rα specifically on FoxP3+ Tregs results in unrestrained lung airway hyperresponsiveness and allergic airway disease, due to reduced suppressive function of Tregs specifically on type 2 ILCs.
Results

IL-4Rα signalling on Tregs modulates expansion and the functional stability of CD4+ CD25+
FoxP3+ Tregs in vivo during allergic disease.

The Foxp3
cre IL-4Rα
lox mouse strain has been previously characterised on the BALB/c background by our laboratory (27) and shown to have an impaired expression of IL-4Rα on CD4+ CD25+ FoxP3+ Tregs in both lung and mediastinal lymph node (mLN) tissue (27). Following the induction of HDM induced allergic inflammation (Figure 1A), we show by flow cytometry (gating strategy, Supplemental Figure 1) a similarly significantly reduced median fluorescence intensity (MFI) on the expression of IL-4Rα subunit on CD4+ CD25+ FoxP3+ Tregs in the lung and mLN tissue in Foxp3
cre IL-4Rα
lox mice compared to IL-4Rα
lox littermate control mice, confirming effective deletion of the receptor (Figure 1B-C). There was upregulation IL-4Rα expression in mLN Tregs in IL-4Rα
lox mouse sensitised and challenged with HDM compared to saline treated control mice (Figure 1C). This suggested that HDM allergic inflammation also induced IL-4Rα signalling on Tregs in mLN. Interestingly, we did not observe a massive induction of IL-4Rα expression by lung Tregs upon HDM challenge, which might suggest different dynamics in occurring in secondary lymphoid tissues and site of disease.

Deletion of IL-4Rα in Foxp3
cre IL-4Rα
lox mice resulted in significantly impaired expansion of CD4+CD25+Foxp3+ Tregs in both mLN and lungs compared to littermate control mice (Figure 1D and E). Although Foxp3 gene is not required for development of Tregs, it is necessary for their maintenance and stability (2, 31). We observed similar expression levels in FoxP3 in mLN Tregs in Foxp3
cre IL-4Rα
lox mice compared to IL-4Rα
lox controls treated with HDM, indicating a maintained Treg stability in the lymphoid tissue (Figure 1E). Interestingly, there was a significant reduction in FoxP3 expression in Tregs in Foxp3
cre IL-4Rα
lox mice when compared...
to littermate control Tregs (Figure 1E), highlighting a possible Treg instability in the local tissue. These findings suggest that IL-4Rα signalling in Tregs regulate expansion of Tregs in periphery and results in impaired expansion and functionality in the local lung tissue during airway inflammation.

IL-4Rα–responsive CD4+ CD25+ FoxP3+ T regulatory cells are required to control airway hyperresponsiveness and mucus hyperplasia in HDM-induced airway inflammation.

STAT6 and IL-4 expression in vivo suppress Tregs during allergic lung inflammation and is thought to dampen T reg suppressive function (32, 33). We then sort to determine whether lack of IL-4Rα signalling on Tregs would result in increased inflammation during HDM model due to reduced suppressive function. We found that HDM sensitisation and challenge of Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> mice, resulted in significantly increased airway resistance and elastance compared to IL-4Rα<sup>−/lox</sup> mice and PBS treated control mice (Figure 2A). This result demonstrated that deletion of the IL-4Rα on Tregs exacerbated AHR. We measured mucus secretion in lung tissue sections and observed a significant increase in mucus production in Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> mice compared to IL-4Rα<sup>−/lox</sup> littermate control mice (Figure 2B). The increased airway obstruction correlated with modest increases in cellular infiltration in BAL fluid and lung tissue of Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> mice compared to IL-4Rα<sup>−/lox</sup> littermate control mice (Figure 2C). We found similar cellular infiltration around the peribronchiolar and perivascular areas of H&E stained lung tissue sections, with PBS control showing little inflammation (Figure 2B). Interestingly, eosinophil frequencies were increased in lung tissue of Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> mice compared to IL-4Rα<sup>−/lox</sup> mice (Figure 2D). Moreover, we observed an accompanying increase in the proportions of CD4+ T cells in lung tissue of Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> mice compared to IL-4Rα<sup>−/lox</sup> littermate control mice, suggestive of an impaired TH cell suppression by Tregs.
(Figure 2D). The effector phenotype on TH cells was maintained along with the intrinsic IL-4Rα signalling in these cells (Supplemental Figure 2A and B). Interestingly, no expansion of CD4 T cells was observed in mLN (Figure 2E), further suggestive of a localised effect on suppressive function. The results clearly highlight an exacerbation in airway hyperreactivity, mucus secretion and lung eosinophilia in IL-4/IL-13 unresponsive Tregs.

We then measured serum levels of HDM-specific IgG1, IgG2a, IgG2b, IgE and total levels of IgE and found similar levels of IgG1 and total IgE in Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox mice and IL-4Rα^{lox} littermate control mice (Figure 3A and B). We found a slight increase in HDM-specific IgG2b and IgG2a levels in IL-4Rα^{lox}\text{-}lox mice compared to Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox mice and PBS control mice and these levels reached significance in IgG2a (Figure 3C). There was a modest reduction (i.e. not in all dilutions) in HDM specific IgE production and a significant decrease in HDM-specific IgG2a in Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox mice compared to IL-4Rα^{lox}\text{-}lox mice (Figure 3A). Overall, these results showed a persistent type 2 antibody production in the absence of IL-4Rα signalling on Tregs during HDM-induced allergic asthma.

*Altered TH2 immunity in lymphoid and local tissue of Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox mice upon acute HDM challenge.*

Crucial in the induction of AHR and mucus hyperplasia are IL-4 and IL-13 cytokines (34). We measured TH2 cytokine production in both HDM and anti-CD3 re-stimulated mLNs (Figure 4A and B). We found a significant reduction in HDM-specific IL-5 and IL-13 and modest increase in IL-4 production in Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox mice compared to IL-4Rα^{lox}\text{-}lox littermate control mice (Figure 4A). We observed similar findings in anti-CD3 re-stimulated mLN particularly in IL-5 and IL-13, however these levels did not reach statistical significance (Figure 4B). Regulatory HDM-specific IL-10 and TH1-associated IFN-γ cytokines were increased in Foxp3^{cre}\text{-}IL-4Rα^{lox}\text{-}lox
mice compared to IL-4Rα/lox littermate control mice. However, we did not observe similar increase in these cytokines upon anti-CD3 re-stimulation (Figure 4B). We assessed intracellular cytokine production in CD4+ T cells using flow cytometry in lungs (Figure 4C-D and Supplemental Figure 3) and mLN s (Supplemental Figure 3). We observed significant reduction in frequencies of CD4+ T cells producing IL-4, IL-13, IL-10 and IL-17 (Figure 4C-D and Supplemental Figure 3), but no changes in total numbers of CD4 T cells producing IL-4, IL-13, IL-10, IL-17 and IFN-γ (Supplemental Figure 3). Our findings so far suggest that in mLN s, TH2 cytokines may be suppressed by a strong induction of IL-10 and IFN-γ, while in the lung reduced TH2 induction may be independent of IL-10 and IFN-γ. Interestingly, despite these slight decreased frequencies in T cell-derived and TH2-associated cytokines (Figure 4A-C), the disease pathology showed signs of being increased in IL-4Rα-unresponsive T reg group. This led us to think that there may be other regulators that contribute towards a significant increase in AHR and pathology and that these regulators may be tissue specific as suggested recently (35).

IL-4-responsive CD4+CD25+FoxP3+ T regulatory cells regulate IL-33 production and ILC2 activation in the Lung.

In the lung, ILC2s and natural killer T cells can secrete type 2 cytokines causing pathology and AHR (36–38). We then changed our focus and looked at the bronchioalveolar (BAL) fluid and lung, which are sites of inflammation and pathology. We started off by measuring TH2 cytokine production in BAL fluid (Figure 5A). We saw similar levels of IL-4 in the BAL fluid when comparing Foxp3creIL-4Rα/lox mice and IL-4Rα/lox littermate control mice (Figure 5A).
Interestingly, we observed a significant increase in total IL-5, IL-13 levels in BAL fluid, along with innate cytokine, IL-33 in Foxp3^{cre}\text{-IL-4R}^{-/}\text{lox} mice compared to IL-4R^{-/}\text{lox} littermate control mice (Figure 5A). This suggested to us that perhaps local cytokine milieu may be what is driving increased pathology and that IL-33 may be a key mediator, as it is a key amplifier of mucosal and systemic innate responses (39).

Type 2 Innate lymphoid cells are recognised as the major producers of IL-5 and IL-13 in lung tissue and are mainly activated by IL-33 (37–40). We then sort to investigate this T cell-independent type 2 increase and how IL-4Rα–responsive CD4^+ CD25^+ FoxP3^+ Tregs could be modulating it. In line with an increase in total IL-33 levels in the BALF (Figure 5A), a corresponding significant increase in ILC2 expansion was observed in Foxp3^{cre}\text{-IL-4R}^{-/}\text{lox} mice compared to IL-4R^{-/}\text{lox} littermate control mice (Figure 5B). Conversely, the increase in ILC2 cells in the lung was complemented by an increase in ILC2s producing IL-5 and IL-13 intracellular cytokines in Foxp3^{cre}\text{-IL-4R}^{-/}\text{lox} mice compared to IL-4R^{-/}\text{lox} littermate control mice (Figure 5C). This result highlights the regulatory role of IL-4Rα- responsive Tregs in local lung tissue inflammation, particularly in suppressing ILC2 derived type 2 inflammatory cytokines during HDM induced allergic airway inflammation.

Epithelial cells secrete IL-33 as an alarmin to any barrier damage during airway inflammation particularly by proteases (41). We observed increased proportions of epithelial cells in Foxp3^{cre}\text{-IL-4R}^{-/}\text{lox} mice when compared to littermate control IL-4R^{-/}\text{lox} mice (Figure 5D). We observed significantly increased IL-33 expression by lung epithelia cells in Foxp3^{cre}\text{-IL-4R}^{-/}\text{lox} mice compared to IL-4R^{-/}\text{lox} mice littermate control, corroborating increased ILC2 induction (Figure 5E). Interestingly, the increase in proportion of lung epithelia cells in IL-4Rα-deficient Tregs was not accompanied by an increase in proliferative capacity as measured Ki67 staining,
but likely due to increase in goblet cells or general epithelial damage (Figure 5F and Figure 2B). Taken together these results demonstrate the importance of IL-4Rα-responsive CD4+CD25+FoxP3+ Tregs in modulating innate cytokine production during acute lung inflammation.

IL-4Rα signalling modulates IL-10 production in the lung CD4+CD25+FoxP3+ T reg compartment.

Previously, disruption of Treg stability by pro-inflammatory cytokines in the lung has been shown to result in an unrestrained TH2 inflammatory response and a reprogramming of Tregs into TH2-like cells, thus exacerbating inflammation (18, 26). We therefore assessed the expression of GATA3 (a TH2 transcription factor) and ST2, an IL-33 receptor subunit to identify a TH2-like reprogramming within FoxP3+Tregs. We observed similar levels of expression in Gata3 in both mLN and lung tissue in FoxP3+Tregs (Figure 6A and B). However, a significant increase in ST2+ Tregs was observed in mLN, but not lungs of Foxp3creIL-4Rαlox/lox mice compared to IL-4Rαlox/lox littermate control mice (Figure 6A and B). Although, the ST2 expression was increased in mLN FoxP3+ Tregs of Foxp3creIL-4Rαlox/lox mice, this did not correlate with IL-13 expression (Figure 6B, Supplemental Figure 4B). Recently, ST2+ Tregs have been shown to secrete IL-13 to reshape the myeloid compartment and control local inflammation after lung injury (42). So, we looked further into these ST2+ Tregs cells in the lung and found them to have impaired IL-13 production in Foxp3creIL-4Rαlox/lox mice compared to IL-4Rαlox/lox mice (Figure 6C, Supplemental Figure 4A). We also observed a significant reduction in Arg1+ myeloid cells (reparative myeloid cells) in Foxp3creIL-4Rαlox/lox mice compared to IL-4Rαlox/lox littermate control mice indicating an altered myeloid compartment and reduced regulation of epithelial damage (Supplemental Figure 6A and B) (42).
ST2+ Tregs are identified as highly activated, strongly suppressive cells with a TH2 bias mainly in non-lymphoid tissues and their suppressive capacity is mediated by IL-10 production (43). The lung ST2+ Tregs had significantly impaired FoxP3 expression and IL-10 secretion in Foxp3<sup>cre</sup>IL-4Rα<sup>-lox</sup> mice compared to IL-4Rα<sup>-lox</sup> littermate control mice confirming an altered regulatory function (Figure 6C, Supplemental Figure 5A). Interestingly, ST2+ Treg population in mLN had comparable expression levels of FoxP3 and IL-10 between Foxp3<sup>cre</sup>IL-4Rα<sup>-lox</sup> mice and IL-4Rα<sup>-lox</sup> mice suggestive of a maintained regulatory potency (Figure 6D, Supplemental Figure 5B) Here, we show an important function of IL-4Rα-responsive FoxP3 T regs in the stability of ST2+ Tregs particularly in their suppressive function during HDM-induced allergic airway inflammation.
Discussion

In this report, we demonstrate that lack of IL-4Rα signalling on Tregs in the context of HDM-induced allergic asthma results in uncontrolled lung pathology and airway hyperreactivity. We employed a murine model where we deleted IL-4Rα on FoxP3+ T regs and found unexpected findings, where deficiency in IL-4Rα on Tregs highlighted a context specificity in regulatory function. We elucidate that FoxP3, a marker of T reg function was maintained in secondary lymphoid tissue, but was severely disrupted in the lung, a site of allergic inflammation. Interestingly, we did not observe hyper activation of TH2 cells, but rather a dysregulated type 2 innate responses mainly driven by epithelium derived IL-33. Of significant interest, IL-4Rα-signalling on FoxP3+ T regs was essential in the accumulation of lung ST2+ T reg cells required for secreting suppressive IL-10 and IL-13, required for promoting reparative arginase expressing myeloid cells. Our results highlight a complex function of IL-4Rα-signalling on FoxP3 T regs in regulating airway hyperreactivity and lung pathology during HDM-induced allergic asthma.

A common role for T reg cells is in controlling the adaptive allergen-specific TH2 immune response (4, 17, 44). Moreover, deficiencies in Treg numbers or Treg trafficking in local tissue leads to an unrestrained allergen-specific TH2 immune cell response(4, 32, 45–47). We recently showed in a helminth model an exacerbated TH2 immune response in mice deficient of IL-4Rα on FoxP3 Tregs (27). However, in HDM-induced allergic asthma we did not observe this unrestrained TH2 inflammatory response, but rather an innate type 2 response. Although T cells commonly contribute to the severity of asthma, they appeared to play a non-significant part in the exacerbation of airway hyperreactivity and remodelling in the absence of IL-4Rα signalling on Tregs. Pillemer and colleagues reported on Tregs being able to inhibit allergen
sensitization and consequently HDM-induced airway inflammation regardless of their responsiveness to IL-4 (25). Our study similarly shows a maintained ability of T cells to produce pro-inflammatory TH2 cytokines (25). In the mLN, there were generally reduced allergen-specific cytokines in the absence of IL-4Rα on FoxP3 T regs, but these did not seem to originate from T cells. FoxP3 expression which we used a proxy for Treg suppressive ability, was maintained in mLN and we speculate is a results of an increase in potent regulatory ST2-expressing Tregs with intact IL-10 secretion (43, 48). However, local lung tissue T regs were severely disrupted in the absence of IL-4Rα on FoxP3 T regs shown by reduced ability to secrete regulatory IL-10 and reduced levels of ST2+ T regs. This is consistent with previous studies where lack of ST2 on FoxP3 T regs resulted in reduced accumulation of intestinal T regs and their ability to adapt to tissue microenvironment and suppress intestinal inflammation (49).Suppressive IL-10 secretion is an integral part of T reg suppressive ability (4, 13) and ST2+ T regs also require IL-10 for their suppressive function (49). Our results were consistent with these findings and also demonstrated reduced ability of both T regs and CD4 T cells to secrete IL-10 (13). Recently, the ST2+ Tregs have been shown to be key in maintaining barrier immunity by secreting IL-13 which modulates the regulatory Arg1+ myeloid cells to repair epithelial damage (42). Our study shows that in the absence IL-4Rα signalling on FoxP3 Tregs there is impaired IL-13 secretion by ST2+ Tregs which control epithelial repair.

T regs cells have been shown to have a propensity to be highjacked and re-programmed under TH2 microenvironment where they lose their FoxP3 and gain TH2-associated transcriptional factors. In these settings, they lose their suppressive function and increase the pool of TH2 cells which can contribute to disease pathogenesis (20, 24, 26). In our setting, we did not observe a gain in GATA3 expression upon loss of FoxP3 in the absence of IL-4Rα signalling on
Tregs. Our studies partly contrast earlier findings which showed a re-programming of FoxP3 Tregs into ex-FoxP3 TH2-like cells during HDM-induced allergic asthma (26). Interestingly, this reprogramming of FoxP3 cells had a modest impact on FoxP3 in both lung and mLN, but reduced BAL fluid eosinophilia, a phenotype we also observed in our model (data not shown). In addition, we did not observe any major changes in antigen-specific antibody production when IL-4Rα signalling on Tregs was disrupted, which validated previous findings that showed no altered antibody responses in the absence of IL-4Rα signalling on Tregs (26, 50). Our findings corroborate other studies where IL-4-deficient Tregs demonstrated incompetency and inferior Treg-mediated immune suppression for both antigen-specific and non-specific T cell activation (33).

T reg cells show tissue specificity during inflammation, especially at mucosal sites and often adapt to local environmental cues which enhances their suppressive function (29, 30). IL-4-responsiveness by FoxP3 Tregs influences their ability to maintain barrier immunity through their regulatory function of ILC2 activation during allergic disease (11, 51). Here, we show an immunoregulatory role by IL-4Rα-responsive Tregs in modulating ILC2 expansion and function in vivo, revealing an important checkpoint for controlling ILC2-mediated type 2 immune responses. This marked ability of iTreg cells to regulate the communication between epithelial cells and ILC2s is critical in acute and chronic disease (52). The mechanism of modulating the innate cellular response involves restraining of epithelial-derived IL-33 secretion in lung tissue. This in turn blocked IL-33 induced ILC2 expansion, rich in IL-5 and IL-13 cytokines which drive recruitment of eosinophils, activation of mast cells and basophils in the local tissue (53, 54). The crucial network of this interaction between epithelial cells and ILC2 generates sustained IL-33 production which is key in the development of persistent/chronic asthma (52).
Sustained barrier disruption induced by HDM proteolytic Derp-1 activity leads to increased IL-33 cytokine production (55, 56). We speculate that both IL-10+ Tregs and T cell derived IL-10 may be critical in suppressing ILC2 activation (57). This idea would be consistent with previous studies where Treg derived-IL-10 was able to suppress ILC2 driven papain-induced airway inflammation via an IL-33/Mast cell axis. It is also likely that there is a direct Treg-ILC2 contact via adhesion molecules such as (ICOS)–ICOS ligand which have been shown to block ILC2 proliferation and type 2 cytokine secretion (11, 44).

Results reported here highlight a complex scenario in the function of IL-4 responsive Tregs whereby they act in suppressing innate type 2 lymphoid cells through IL-10 production and controlling IL-33-mediated allergic inflammation. The local inflammatory environment is crucial in providing local cues that dictate Treg expansion and function. In this context IL-4Rα-responsive Tregs show incredible specificity in the lung and directs suppression of lung ILC2s, but not TH2 cells. We therefore provide evidence for the indispensable role of IL-4Rα signalling in maintaining Treg stability and IL-4-responsive Tregs in limiting IL-33 derived ILC2 driven AHR and airway inflammation in HDM induced allergic asthma. Further studies are necessary to elucidate the exact mechanisms of epithelial immune modulation by Tregs and the lung specific cues modulating the Treg stability during type 2 airway immune responses. Our study also places caution to anti-IL-4Rα therapy which may have undesirable interference in antigen-specific Treg based tolerance (25) which we show to be key in restraining IL-33-mediated allergic inflammation.
Methods

Mice. Transgenic Foxp3<sup>Cre</sup> mice (a generous gift from Prof. James Wing, Osaka University, Osaka, Japan) on BALB/c background were intercrossed for two generations with IL-4Rα<sup>−/−</sup> BALB/c mice (58) to generate a Foxp3<sup>Cre</sup>IL-4Rα<sup>−flox</sup> (27), IL-4Rα<sup>−flox</sup> (59) hemizygotes on a BALB/c background were backcrossed up to 10 generations. All mice were used at eight to 10 weeks of age. Mice were housed in independently ventilated cages under specific pathogen-free conditions in the University of Cape Town Animal Facility.

House Dust-mite induced allergic airway disease. Mice were anaesthetised with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa) and xylazine (Rompun; Bay, Isando, South Africa) and sensitised intratracheally (i.t.) with 1µg of HDM (Stellergens Greer Laboratories, Lenoir, U.S.A.) on day 0. Mice were intranasally (i.n.) challenged with 10µg of HDM under anaesthesia on days 6, 7, 8, 9 and 10 as previously described (60). AHR was measured on day 14. After the procedure, mice were euthanised and tissue samples were taken for analysis.

Airway Hyperresponsiveness. Airway resistance and elastance of the whole respiratory system (airways, lung chest wall) after intranasal challenge was determined by forced oscillation measurements as described previously(61) with the Flexivent system (SCIREQ, Montreal, Canada) by using the single compartment (‘’snapshot’’) perturbation. Measurements were carried out on mice with increasing doses of acetyl-β-methylcholine (methacholine, Sigma-Aldrich, Aston Manor, South Africa) treatment. Differences in the dose-response curves were analysed by repeated-measures ANOVA with the Bonferroni post-test. Only mice with
acceptable measurements for all doses (coefficient of determination >0.90) were included in the analysis.

Flow cytometry. Bronchoalveolar lavage (BAL) fluid cells were obtained as previously described(62). Single-cell suspensions were prepared from lymph nodes in Iscove’s Modified Dulbecco’s Medium (IMDM) (Gibco, Paisley, United Kingdom) by passing them through a 100µm filter. To obtain single cell suspensions from lung tissues, a left lobe lung was digested for 1 hour at 37°C in RPMI (Gibco, Paisley, United Kingdom) containing 13 mg/mL DNase I (Roche, Randburg, South Africa) and 50 U/mL collagenase IV (Gibco, Waltham, Massachusetts) and passed through a 70µm filter. IL-4Rα surface expression was detected on lymph node cells, lung cells and BAL fluid cells by phycoerythrin (PE) anti-CD124 (IL-4Rα, M-1). Antibodies used in these experiments included (Table 1), phycoerythrobilin (PE)-conjugated anti-Siglec-F (clone, E50-2440), anti-CD124 (IL-4Rα, clone, M-1), anti-IL-5 (clone, TRFK5), anti-Ki67 (clone, B56), anti-CD44 (clone, KM114), FITC-conjugated anti-Gr-1 (clone, RB6-8C5), CD45 (clone, 30-F11), IL-4 (clone, 11B11), PerCP Cy5.5-conjugated anti-Ly6C (clone, AL-21), -CD45.1 (clone, A20), anti-IL-17 (clone, TC11-18H10), Allophycocyanin (APC)-conjugated anti-CD11c (clone, HL3) -anti-IL-10 (clone, JESS-16E3), anti-FoxP3 (clone, MF23), V450 conjugated anti-CD11b (clone, M1/70), anti-CD62L (clone, MEL-14), AlexaFlour 700-conjugated anti-CD3ε (clone, 145-2C11) -anti IFN-γ, V500- anti-CD4 (clone, RM4-5) and anti-B220 (clone, RA3-6B2), APC-Cy7-conjugated anti-CD19 (clone, 1D3), anti-EpCAM (clone, G8.8) and anti-CD8 (clone, 53-6.7), biotin-CD25 (clone, 7D4), PerCP Cy5.5 Lineage antibody cocktail, were purchased from BD Biosciences, PE-Cyanine7 anti-F4/80 (clone, BM8), anti-IL-13 (clone, eBio13A), AlexaFlouro 700- conjugated anti-MHC II (clone, M5/114) and Live/dead Fixable Yellow stain (Qdot605 dead cell exclusion dye) were purchased from eBiosciences,
Arginase (Arg1), FITC-anti-SCA-1 (clone, D7), PE-Cy7 anti-GATA3 (clone, L50-823). FITC anti-T1/ST2 (clone, DJ8) was purchased from md bioproducts. PE-anti IL-33 (clone, 396118) was purchased from Invitrogen. Biotin-labelled antibodies were detected by Texas Red conjugated PE (BD Biosciences). For staining, cells (1x10^6) were labelled and washed in PBS, 3% FCS FACS buffer. For intracellular cytokine staining, cells were restimulated with phorbol myristate acetate (Sigma-Aldrich) (50 ng/mL), ionomycin (Sigma-Aldrich) (250ng/mL), and monensin (Sigma-Aldrich) (200mM in IMDM/10% FCS) for 6h at 37˚C then fixed in 2% PFA, permeabilised and cytokine production was analysed on a LSR Fortessa machine (BD Immunocytometry system, San Jose, CA, USA) and data were analyzed using Flowjo software (Treestar, Ashland, OR, USA). using Flowjo. All antibodies were from BD Pharmingen (San Diego, CA).

**Histology.** Lungs were fixed in 4% formaldehyde/PBS and embedded in paraffin. Tissue sections were stained with periodic acid-Schiff for mucus secretion, and haematoxylin and eosin (H&E) staining for inflammation. Image analysis was performed on NIS Elements (Nikon Instruments, Tokyo, Japan). Mucus quantification was carried out using the automated NIS Elements software by defining regions of Interest (ROIs) which are the individual bronchioles on cut lung sections to be analysed for mucus staining and using threshold quantification of the mucus stain in the specific ROIs NIS Elements (Nikon Instruments, Tokyo, Japan). Area of staining is defined as total area of mucus secretion per area of bronchiole epithelial lining. Lung sections from individual mice were assessed, and data from 3 experiments were pooled (n = 4-6 mice per experiment, 27-57 airway bronchioles analysed/group).

**Antibody and cytokine ELISAs.** Antibody ELISAs were carried out as previously described (62) using 10 µg/ml HDM to coat for specific IgE and 5 µg/ml HDM to coat for specific IgGs. For in
in vitro cytokine production analysis, single cell suspensions were prepared from mediastinal lymph nodes of HDM-treated and littermate control mice (62). Cells (2\times 10^5 cells, in 200\mu L) were incubated for 5 days in IMDM/10% FCS (Delta Bioproducts, Kempton Park, South Africa) in 96-well plates. Cells were either stimulated with HDM (30\mu g/mL) or anti-CD3 (10\mu g/mL) and supernatants were collected after a 5-day incubation period. BAL cells were isolated from BALF and lungs were homogenized, and supernatant assessed for cytokine production after quantifying the protein concentration using BCA kit (ThermoFisher, Rockford, USA).
Concentrations of IL-4, IL-5, IFN-\gamma, (BD Biosciences), IL-13, IL-33 (R&D Systems, Minneapolis, Minn), IL-17 and IL-10 (BioLegend) were measured using ELISA assays according to the manufacturer’s protocol.

Statistics. P values were calculated in GraphPad Prism 6 (GraphPad Software, Inc) by using nonparametric Mann-Whitney Student's t-test, One-way ANOVA with Tukey’s test for multicomparison or Two-way ANOVA with Bonferroni's post-test for multiple comparisons, and results are presented as standard error of the mean (SEM) or mean of standard deviation (SD). Differences were considered significant if P was <.05.

Study approval. Animal procedures were performed according to strict recommendation by the South African Veterinary Council and were approved by the University of Cape Town Animal Ethics Committee (Reference number 014/019 or 018/013).
**Author contributions.** Conceived and supervised study: FB FK SH. Performed the experiments: JK SH FK. Analysed the data: JK SH FB. Wrote the paper: JK FB SH. All authors discussed the results and commented on the manuscript.

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42. Liu Q et al. IL-33–mediated IL-13 secretion by ST2+ Tregs controls inflammation after lung injury. JCI Insight 2019;4(6):e123919.


50. Aziz NA, Komguep J, Id N, Mpotje T, Brombacher F. The Foxp3+ regulatory T-cell population requires IL-4Rα signaling to control inflammation during helminth infections [published online ahead of print: 2018]; doi:10.1371/journal.pbio.2005850.


Figure 1. IL-4Rα unresponsive CD4^+CD25^+FoxP3^+ T regulatory cells results in impaired T reg function mainly in the lung. (A) Schematic of the HDM induced airway inflammation model, where Foxp3^{cre-IL-4Rα/lox} and IL-4Rα^{lox/lox} mice were sensitised with HDM 1 µg at day 0 intratracheally and challenged with HDM 10 µg at day 6-11. AHR and FACS analyses was done at day 14. (B) Flow cytometry histograms of Treg (CD3^+CD4^+CD25^+FoxP3^+) IL-4Rα expression in mediastinal lymph nodes (mLN) and lung tissues. (C) Quantification of Treg (CD3^+CD4^+CD25^+FoxP3^+) IL-4Rα expression in mLN and lung tissue represented as MFI. Scatter plot represent mean ± SD from 1 representative experiment of 3 independent experiments (IL-4Rα^{lox/lox} PBS n=3, IL-4Rα^{lox/lox} HDM n=6, Foxp3^{cre-IL-4Rα/lox} HDM n=6, IL-4Rα^{+/−} HDM n=5). (D) Proportion of CD4^+ CD25^+ FoxP3^+ T reg in mLN and lung tissue. Scatter plot represent mean ± SD from 1 representative experiment of 3 independent experiments (IL-4Rα^{lox/lox} PBS n=3, IL-4Rα^{lox/lox} HDM n=6, Foxp3^{cre-IL-4Rα/lox} HDM n=6, IL-4Rα^{+/−} HDM n=5). (E) Flow cytometry histograms of FoxP3 expression on CD4^+ CD25^+ FoxP3^+ T reg in mLN and lung tissue. Numbers shows quantitative MFI mean ± SEMs (IL-4Rα^{lox/lox} n=8, Foxp3^{cre-IL-4Rα/lox} n=5).
Significant differences are described as: *p < .05, **p < .01, ***p < .001, ****p<.0001 and were performed using One-way ANOVA with Tukey's multicomparison test. MFI, Median fluorescence intensity; FMO, fluorescent minus one; ns, not significant.

**Figure 2**
Figure 2. Deletion of IL-4Rα on CD4^+CD25^+FoxP3^+ T regs exacerbates airway hyperresponsiveness and mucus hyperplasia in HDM airway inflammation. (A) Airway resistance and Airway elastance were measured using Flexivent by comparing dose-response curves to inhaled methacholine (0-40 mg/mL). Significant differences between control saline mice (n=7), Foxp3^cre^IL-4Rα^lox^ mice (n=10) and IL-4Rα^lox^ mice (n=7) are described as: *p < .05, **p < .01 and were performed using Two-way NOVA with Benferroni post-test. Data representative mean ± SEMs from 2 pooled experiments. (B) Histology of lung sections stained with periodic acid shift (PAS) or haematoxylin and eosin (H&E). Automated quantification of the area (µm^2) of mucus staining per analysed bronchiole was carried out using NIS elements imaging software. PAS sections are magnified to 200x and H&E slides are magnified to 40x. Scatter plots show measurement of PAS area mean ± SEMs (control saline mice n=27, IL-4Rα^lox^ n=57, Foxp3^cre^IL-4Rα^lox^ n=56). (C) Total cellular infiltration in bronchoalveolar lavage fluid and lung cells. (D) Number of lung eosinophils (live, CD11c^low^CD11b^high^Ly6G^low^SiglecF^hi^) and CD4 T cells (live, CD3^+CD8^CD4^+) analysed by flow cytometry. (E) Number and frequencies of mediastinal lymph node CD4^+^ T cell (live, CD3^+CD8^CD4^+) analysed by flow cytometry. Scatter plots (C-E) show mean ± SD from 1 representative experiment of 3 independent experiments (IL-4Rα^lox^ PBS n=5, IL-4Rα^lox^ HDM n=5, Foxp3^cre^IL-4Rα^lox^ HDM n=8). Significant differences are described as: *p < .05, **p < .01, ***p < .001, ****p<.0001 and were performed using One-way ANOVA with Tukey's multicomparison test.
Figure 3. Deletion of IL-4Rα on CD4<sup>+</sup>CD25<sup>+</sup>FoxP3<sup>+</sup> T regs does not alter antibody production during HDM induced allergic asthma. (A) HDM-specific IgG and HDM-specific IgE. (B) Total IgE. (C) HDM-specific IgG2a and HDM-specific IgG2b measured by ELISA. Data shown (A-C) is mean ± SD from 1 representative experiment of 3 (IL-4Rα<sup>lox/lox</sup> PBS n=3, IL-4Rα<sup>lox/lox</sup> HDM n=5, Foxp3<sup>cre</sup>IL-4Rα<sup>lox/lox</sup> n=5). Significant differences are described as: *p < .05, **p < .01, ***p < .001, ****p<.0001 and were performed using One-way ANOVA with Tukey’s multicomparison test.
Figure 4. Impairment of HDM specific TH2-associated cytokine production in peripheral lymphoid, but not local lung tissue of FoxP3<sup>cre</sup>IL-4Rα<sup>lox/lox</sup> mice during HDM induced airway inflammation. TH2 associated cytokine production were measured in a 5 day <i>ex vivo</i> re-stimulated mediastinal lymph nodes (mLN) using ELISA. (A) HDM re-stimulated mLNs. Scatter plot represent mean ± SEM of 3 pooled experiments (IL-4Rα<sup>lox/lox</sup> n=16, Foxp3<sup>cre</sup>IL-4Rα<sup>lox/lox</sup> n=17). (B) anti-CD3 re-stimulated mLN. Scatter plot represent ± SEM of 3 pooled experiments (IL-4Rα<sup>lox/lox</sup> n=16, Foxp3<sup>cre</sup>IL-4Rα<sup>lox/lox</sup> n=17). (C) Flow cytometry plots for CD4<sup>+</sup> T cells producing intracellular IL-4, IL-13 and IFN-γ after 5 hr stimulation with PMA/ionomycin.
and monensin. (D) Scatter plot frequencies of cytokine producing CD4⁺ T cells based on percentages shown in C. Data shown is mean ± SDs 1 representative experiment of 3 independent experiments (IL-4Rα⁺/⁻ n=4, Foxp3⁺/IL-4Rα⁺/⁻ n=7). Significant differences between groups are represented as *p < .05 and were performed using Mann-Whitney student’s t-test.
Figure 5. Exacerbated innate type 2 cytokine production in lung of Foxp3\textsuperscript{cre}IL-4R\textalpha/\textsuperscript{lox} mice upon acute HDM challenge. (A) BAL cytokines (IL-4, IL-5, IL-13 and IL-33) were measured by ELISA and corrected to the protein concentration. Scatter plot represent mean ± SEM of 2 pooled experiments (IL-4R\textalpha/\textsuperscript{lox} n=10, Foxp3\textsuperscript{cre}IL-4R\textalpha/\textsuperscript{lox} n=12) (B) Flow cytometry plots of
innate type lymphoid cells (ILC2s), Live<sup>+</sup> singlets, lymphocyte, Lineage<sup>-</sup> SCA<sup>+</sup> CD127<sup>+</sup>T1/ST2<sup>+</sup> and quantification in frequency of ST2<sup>+</sup>CD127<sup>+</sup>. Scatter plot represent mean ± SD from 1 representative experiments from 2 independent experiments (IL-4Rα<sup>−/lox</sup> n=6, Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> n=6). (C) Flow cytometry plots and quantification of ILC2s producing intracellular IL-5 and IL-13 after 5 hr stimulation with PMA/ionomycin and monensin. Scatter plot data shown represent mean ± SD from 1 representative experiments from 3 independent experiments (IL-4Rα<sup>−/lox</sup> n=6, Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> n=6) (D) Flow cytometry plots and quantification of epithelial cells (Live, CD45<sup>+</sup>EpCam<sup>+</sup>MHCII<sup>+</sup>) Scatter plot data shown represent mean ± SD from 1 representative experiments from 3 independent experiments (IL-4Rα<sup>−/lox</sup> n=6, Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> n=6) (E) Representative histogram plot and quantification of IL-33 MFI from epithelial cells measured by flow cytometry. Scatter plot data shown represent mean ± SD from 1 representative experiments from 2 independent experiments (IL-4Rα<sup>−/lox</sup> n=6, Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> n=6) (F) Flow cytometry histogram of Ki67 proliferative marker expression of epithelial cells as gated in D and represented as MFI. Scatter plot data shown represent mean ± SD from 1 representative experiments from 2 independent experiments (IL-4Rα<sup>−/lox</sup> n=6, Foxp3<sup>cre</sup>IL-4Rα<sup>−/lox</sup> n=6). Significant differences are described as *p < .05, **p < .01 and were performed using Mann-Whitney student’s t-test. MFI, Median fluorescence intensity; FMO, fluorescent minus one.
IL-4/IL-13-responsive CD4^+CD25^+FoxP3^+ T regulatory cells regulate IL-10 production in the lung. Flow cytometry analysis of GATA3 and ST2 expression within CD4^+CD25^+Foxp3^+ Tregs (A) Lung tissue. (B) Mediastinal lymph node (mLN) tissue. (C) Proportion of ST2^+ Tregs in the lung and expression of FoxP3, IL-10 and IL-13. (D) Proportion of ST2^+ Tregs in the mLN and expression of FoxP3, IL-10 and IL-13. Data shown (A-D) is mean ± SD of 1 representative experiment of 3 independent experiments (IL-4Rα^−/lox n=6, Foxp3^cre IL-4Rα^−/lox n=6). Significant differences among groups are represented as *p < .05, **p < .01 and were performed using Mann-Whitney student’s t-test. MFI, Median fluorescence intensity; FMO, fluorescent minus one.
### Table 1: List of antibodies used for flow cytometry

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