

ORIGINAL ARTICLE

Reciprocal expression of IL-25 and IL-17A is important for allergic airways hyperreactivity

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Summary

Background Interleukin (IL)-25 (IL-17E) is a potent inducer of the type-2 immune effector response. Previously we have demonstrated that a neutralizing anti-IL-25 antibody, given during the establishment of ovalbumin-specific lung allergy, abrogates airways hyperreactivity.

Objective Blocking IL-25 results in the suppression of IL-13, a cytokine known to exacerbate pulmonary inflammation, and an unexpected reciprocal increase in IL-17A. The role of IL-17A in asthma is complex with reports of both pro-inflammatory and anti-inflammatory functions. Our aim was to determine the influence of IL-17A in regulating IL-25-dependent lung allergy.

Method Neutralizing antibodies to IL-25 and/or IL-17A were administered during an experimental model of allergic asthma. Bronchoalveolar cell infiltrates and lung cytokine production were determined to assess lung inflammation. Invasive plethysmography was undertaken to measure lung function.

Results Neutralization of IL-25 correlated with a decrease in IL-13 levels and an increase in IL-17A production, and an accompanying prevention of airway hyperresponsiveness (AHR). Notably, the blocking of IL-17A reversed the protective effects of treating with anti-IL-25 antibodies, resulting in the re-expression of several facets of the lung inflammatory response, including IL-13 and eotaxin production, eosinophilia and AHR. Using mice over-expressing IL-13 we demonstrate that treatment of these mice with anti-IL-25 fails to suppress IL-13 levels and in turn IL-17A levels remain suppressed.

Conclusions and Clinical Relevance IL-13 is known to be an important inducer of lung inflammation, causing goblet cell hyperplasia and promoting airways hyperreactivity. Our data now demonstrate that IL-13 also plays an important role in the genesis of lung inflammation downstream of IL-25 by suppressing a protective IL-17A response. These findings also highlight the important reciprocal interplay of the IL-17 family members, IL-25 and IL-17A, in regulating allergic lung responses and suggest that the balance of IL-17A, together with IL-25, will be an important consideration in the treatment of allergic asthma.

Keywords airways hyperreactivity, asthma, IL-17A, IL-25, IL-13, type-2

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Introduction

It is well established from both human and mouse studies that the classical type-2 cytokines IL-4, IL-5, and IL-13 play a central role in the pathogenesis of asthma and accordingly they have become major targets for developing therapeutics for treatment of allergic airway disease.

Key amongst these type-2 mediators is IL-13 the regulation of which has been shown to be critical for the development of airways hyperreactivity and many of the inflammatory traits that characterize asthma [1–3]. As such, antagonists of IL-13 are being actively pursued as potential therapeutics in asthma [4, 5].

We know many of the effector functions that are initiated by IL-13, for example goblet hyperplasia and mucus production, alternative activation of macrophages,

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fibrosis, IgE expression and increased susceptibility to airway hyperresponsiveness (AHR). More recently we have also started to understand how IL-13 expression is regulated. IL-25, a member of the IL-17 cytokine family, has been shown to play an important role in promoting the efficient onset of type-2 cytokine production both in the innate type-2 response and in adaptive type-2 immunity [6–8]. IL-25 differs from other IL-17 family members in that its production induces type-2 cytokine expression associated with splenomegaly, elevated serum levels of IgG1 and IgE, and pathological changes in the lungs and digestive tract including eosinophilic infiltrates, increased mucus secretion and epithelial cell hyperplasia [9–12]. Genetic ablation of IL-25 or the use of blocking anti-IL-25 antibodies have clearly demonstrated the importance of IL-25 in protecting from helminth infection [7, 8], but also its critical role in regulating responses characteristic of asthma [13]. It appears that IL-25 stimulates these responses through its ability to induce the release of type-2 cytokines initially from innate non-B/non-T cells, termed nuocytes, [6, 7, 9] and subsequently from the adaptive T cell response [14, 15].

Consistent with a role in asthma, IL-25 mRNA or protein has been detected from a number of cell types found in the lung including alveolar macrophages, mast cells, eosinophils, and basophils [9, 14, 16, 17]. More recently, IL-25 production by allergen-stimulated human and mouse lung epithelial cells has supported a potential role for IL-25 modulating allergic pulmonary responses [15]. In addition, IL-25 has been reported to induce inflammatory cytokine and chemokine production from lung fibroblasts [18], and components of extra-cellular matrix from airway smooth muscle cells [18, 19]. Recent studies have also indicated that transcripts for IL-25 and IL-25R are significantly up-regulated in biopsy tissue from asthmatic patients, associated with eosinophilic infiltration [14, 18].

By contrast the other five family members (IL-17A, IL-17B, IL-17C, IL-17D, and IL-17F) are associated with type-1 inflammation. The role of IL-17A in autoimmunity has now been firmly established in the mouse with the identification of T-helper 17 (Th17) cells. Th17 cells appear to have evolved to provide protection against microbes not effectively dealt with by the Th1 and Th2 arms of immunity [20] and IL-17A is critical for host defence against *Klebsiella pneumoniae* [21], *Bacteroides fragilis* [22], and *Candida albicans* [23]. However, the role of IL-17A in asthma is complicated and controversial. IL-17A-deficient T cells, from mice immunized with OVA complexed with alum, proliferated less well *ex vivo* and produced lower levels of IL-4 and IL-5 compared with wild-type T cells, suggesting IL-17A is required for optimal activation of Th2 cells [24]. In addition, mice deficient in IL-17A or IL-17A receptor failed to develop AHR or pulmonary inflammation in an OVA-induced model of

asthma [24, 25]. However, a recent report has suggested that administration of exogenous IL-17A resulted in an improvement of lung function associated with reduced levels of the chemokines TARC, RANTES, and eotaxin, which are involved in the recruitment of Th2 cells, eosinophils, and basophils to the lung [24]. A corresponding decrease was seen in bronchoalveolar lavage (BAL) eosinophils and lymphocytes. Thus IL-17A may play differing roles in asthma responses dependent upon when it is expressed during the induction of allergic responses.

Interestingly, the IL-25 receptor has now been shown to function as a dimeric receptor complex, containing IL-17RA and IL-17BR. Consequently, mice deficient in IL-17RA do not respond to IL-25 [26]. Although IL-17A and IL-25 have divergent functional properties a recent report has suggested that the extent of inflammation observed in experimental autoimmune encephalitis (EAE) is dependent on the balance of IL-25 and IL-17A [25]. Administration of exogenous IL-25 protected wild-type mice from EAE development and reduced Th17 numbers, leading to the hypothesis that IL-25 inhibits differentiation of Th17 cells. Strikingly, IL-25 prevented EAE in *Il4*^{-/-} mice but not in *Il13*^{-/-} or *Il4R α* ^{-/-} mice indicating that IL-25 acts via IL-13 to regulate IL-17A and suppress disease [25].

We recently reported that during a mouse model of experimental asthma treatment with an anti-IL-25 mAb efficiently prevented airways hyperreactivity and lung inflammation [13]. We now demonstrate that IL-25 is a critical regulator of the IL-13/IL-17A axis during pulmonary responses. Inhibiting IL-25 during the challenge phase results in a significant fall in IL-13 levels and a reciprocal increase in IL-17A that we now demonstrate to be essential for protection against airways hyperreactivity. Concurrent neutralization of IL-25 and IL-17A leads to the resumption of AHR. We go on to show that in conditions where inhibition of IL-25 cannot suppress IL-13 secretion IL-17A production fails to increase, indicating that the regulation of IL-17A is via IL-13. Thus, the suppression of IL-17A by IL-13 is critical for inducing experimental allergic asthma.

Materials and methods

Mice

BALB/c mice (Charles River facility, 6–8 weeks) and *Il13*Tg mice [27] back-crossed to BALB/c for more than 10 generations were maintained in a specific pathogen-free environment. All animal experiments were undertaken with the approval of the UK Home Office.

Sensitization, allergen exposure, and measurement of airway hyperresponsiveness

Mice were sensitized by intraperitoneal administration of endotoxin low ovalbumin (OVA) complexed in a 1 : 1 ratio

with alum (Imject[®], Thermo Scientific) and challenged with aerosolized standard grade OVA [13]. Control animals received phosphate-buffered saline (PBS). Twenty-four hours after the final aerosol challenge AHR was assessed using a restrained whole body plethysmograph (EMMS, Bordon, UK). Animals were anaesthetized, tracheostomized, and ventilated (MiniVent 845 ventilator, EMMS) at a rate of 150 breaths/min, with a tidal volume of 150 μ L/stroke. After recording stable baseline total respiratory system resistance for 3 min increasing concentrations of acetyl- β -methylcholine chloride (methacholine) (Sigma-Aldrich, Dorset, UK) were administered by aerosol for 10 s with an ultrasonic nebulizer, and total respiratory system resistance was recorded for a 3-min period. eDaq software was used to analyse airways resistance.

Monoclonal antibody administration

All antibodies used for *in vivo* work were administered via the intraperitoneal route 2 h before each aerosol administration. The mouse IgG1 isotype control antibody and anti-IL-25 neutralizing antibody have been described previously [13] and were used at 500 μ g per dose. The rat IgG2a isotype control antibody (clone 20102) and anti-IL-17A neutralizing antibody (clone 50104) were obtained from R and D Systems (Abingdon, UK) and were used at 7.5 μ g per dose. This dose was based on the inhibition of IL-17A as demonstrated by Schnyder-Candrian et al. [24]. Anti-IL-25 (S. J. Ballantyne, unpublished data) and anti-IL-17A (R and D Systems) have been tested for cross reactivity and do not recognize any other IL-17 family members.

Flow cytometry

Lung cell suspensions, treated with 720 μ g/mL collagenase D (Amersham, Bucks, UK), and BAL cell suspensions were stimulated *ex vivo* with media alone, or 50 ng/mL PMA, 500 ng/mL ionomycin for 4 h at 37 °C. All cells also received 1 μ L/mL of Golgi plug (BD Biosciences, Oxford, UK). Following stimulation all cells were stained for cell surface expression of CD3 and CD19 using anti-CD3 conjugated to FITC (clone 145-2C11, eBioscience, San Diego, CA, USA) and anti-CD19 conjugated to PECy7 (clone eBio1D3, eBioscience). The cells were then fixed, permeabilized, and stained for IL-13 with anti-IL-13 conjugated to PE (clone eBio13A, eBioscience), and IL-17A: anti-IL-17A conjugated to Alexa 647 (clone eBioTC11-18H10.1, eBioscience) according to the manufacturers instructions (BD Biosciences).

Differential white blood cell and bronchoalveolar lavage cell counts

Blood smears or BAL cell cytospin slides were stained with Giemsa (Sigma-Aldrich), following the manufacturer's

instructions. Differential cell counts were made blind on 100 cells per slide under oil and at a $\times 63$ magnification.

Bronchoalveolar lavage cytokine enzyme-linked immunoassay

The IL-5 cytokine ELISA utilized the sandwich format with capture and detection antibodies purchased from BD Biosciences. The ELISA was performed according to BD Biosciences ELISA protocol. The IL-13, IL-17A, eotaxin, and TARC cytokine ELISAs were performed using the appropriate R and D Systems Quantikine ELISA kits according to the manufacturer's instructions.

Lung histology

Lung cells were fixed in formalin (10% formaldehyde in 0.9% saline solution) and stained with Giemsa for inflammatory cell infiltrate and Periodic acid-Schiff for airway mucus production. Inflammation and mucus production was evaluated using a blind numerical scoring system expressed in arbitrary units [28].

Statistical analysis

The significance of the differences between experimental groups was analysed by using a two-way ANOVA with Bonferroni's post-test (for dose-response to methacholine) or Student unpaired *t* test.

Results

Blocking IL-25 results in increased levels of IL-17A that are required to suppress IL-13 secretion

In order to test the effects of blocking IL-17A and IL-25 during an allergic inflammatory lung response, mice were sensitized with two doses of OVA before being challenged with aerosolized OVA. Administration of anti-IL-25 neutralizing antibody during the OVA challenge phase of the allergic response (Fig. 1a) decreased the levels of IL-13 in the BAL fluid (Fig. 1b). By contrast we detected increasing levels of the cytokine IL-17A in the lungs of the anti-IL-25-treated mice (Fig. 1c). To test if the elevated level of IL-17A was playing a mechanistic role in regulating the cytokine and inflammatory response, mice were administered with either neutralizing anti-IL-25 or neutralizing anti-IL-17A antibody alone, or with the two antibodies in combination, during the challenge phase of the OVA-induced experimental asthma model (Fig. 1a). Isotype control antibodies were administered as appropriate. Administration of anti-IL-17A alone resulted in levels of IL-13 equivalent to the isotype control group (Fig. 1b). In contrast to the reduction of IL-13 and increase in IL-17A that was observed following anti-IL-25 mAb treatment,

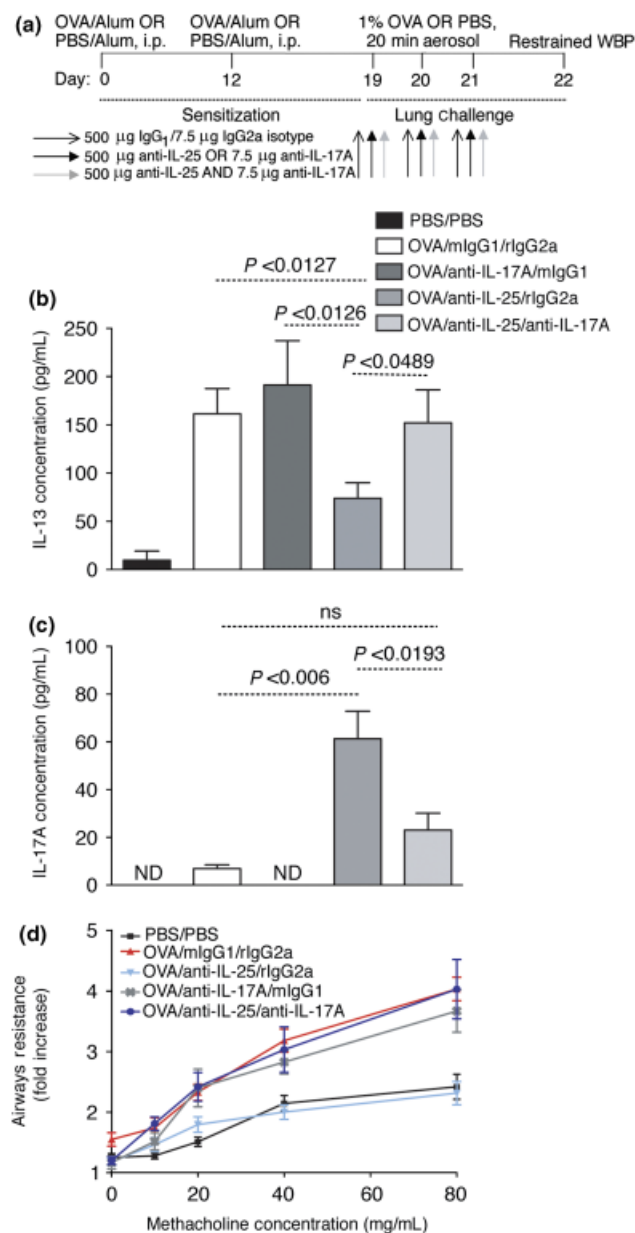


Fig. 1. IL-17A is required for the protective effects of anti-IL-25 in hyperresponsiveness (AHR). (a) Ovalbumin-sensitized and challenged mice were injected with anti-IL-25 and/or anti-IL-17A. Bronchoalveolar lavage fluid was harvested after OVA sensitization and challenge. (b) IL-13 and (c) IL-17A levels were assayed by ELISA. Data are representative of three independent experiments ($n = 5$ mice per group). (d) Airways resistance to increasing concentrations of methacholine. Data are representative of two independent experiments ($n = 10$ mice per group). ND, not detected.

the additional co-administration of anti-IL-17A mAb with anti-IL-25 resulted in recovery in the levels of pulmonary IL-13 (Fig. 1b). These data demonstrate that the elevated levels of IL-17A observed after anti-IL-25 treatment are required to suppress IL-13 production.

The balance of IL-25 and IL-17A regulates ovalbumin-induced airway hyperresponsiveness

To test whether the expression of IL-17A was playing a key functional role we investigated whether the prevention of AHR, as assessed by measuring airways resistance, following administration of anti-IL-25 was dependent on the production of IL-17A. As expected, administration of anti-IL-25 completely prevented AHR (Fig. 1d). Strikingly, blocking the activity of IL-17A in the presence of anti-IL-25 reversed the beneficial effects of anti-IL-25 mAb and resulted in the re-emergence of profound AHR (Fig. 1d). Co-administration of an isotype-matched control antibody did not alter the effects of anti-IL-25, and administration of anti-IL-17A mAb alone had no effect on AHR (Fig. 1d). Thus, the IL-17A expression that arises following neutralization of IL-25 plays a positive role in protecting against AHR. These results clearly demonstrate that the relative levels of IL-17A and IL-25 play critical roles during pulmonary allergic asthma responses.

IL-17A is required to suppress eotaxin production and eosinophilia following IL-25 neutralization

We tested the IL-25/IL-17A cytokine balance further to investigate whether other pulmonary allergic mediators were affected. Anti-IL-25 treatment caused a significant decrease in circulating eosinophil numbers and pulmonary eotaxin (Figs 2a and b). By contrast, the co-administration of anti-IL-17A reversed the effects of blocking only IL-25, giving comparatively similar results as those seen following isotype control antibody treatment (Figs 2a and b). Notably, anti-IL-17A treatment alone caused elevated levels of pulmonary eotaxin and blood eosinophilia compared with animals treated with isotype control antibodies (Figs 2a and b), supporting the hypothesis that IL-25 and IL-17A play opposing roles in the regulation of lung allergy.

In contrast to eosinophilia and BAL eotaxin, production of IL-5 in the BAL fluid, while up-regulated by OVA challenge, compared with PBS-treated mice, was not significantly altered in any of the anti-IL-25/anti-IL-17A treatment groups (Fig. 2c). Similarly, airway mucus production and BAL eosinophils were not affected by IL-25 and IL-17A levels (Figs 2d and e). Additionally, Schnyder-Candrian et al. [24] had previously suggested a role for IL-17A in the regulation of TARC production by dendritic cells, *in vitro* [24]. However we were unable to detect any modulation of TARC following anti-IL-25 and anti-IL-17A administration (Fig. 2f). These results suggest that the opposing effects of IL-25 and IL-17A are only critical for specific aspects of the type-2 response during experimental allergic asthma.

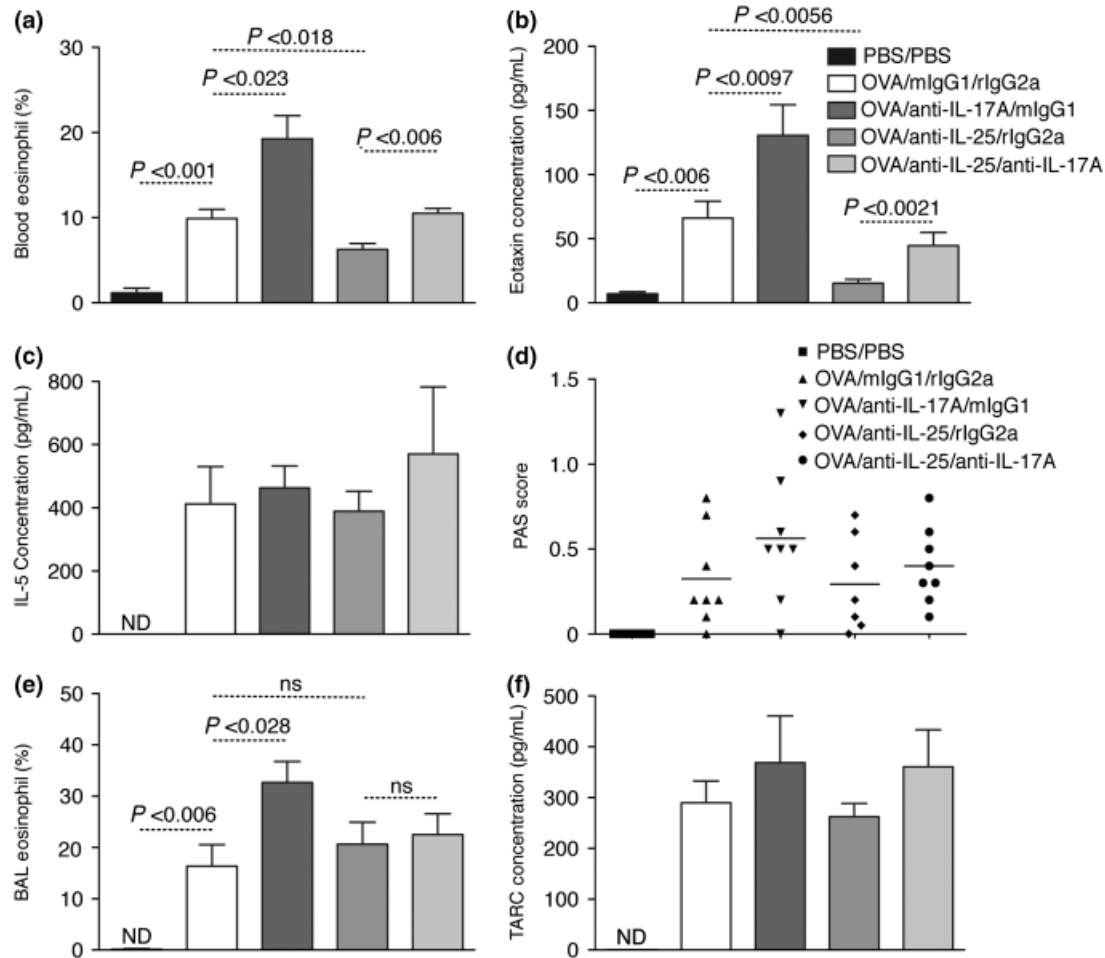


Fig. 2. Eotaxin levels and blood eosinophilia are reciprocally regulated by IL-25/IL-17 levels. (a) Blood smears were made from ovalbumin (OVA) sensitized and challenged mice to assess eosinophilia. Bronchoalveolar lavage (BAL) fluid was harvested after OVA sensitization and challenge and assayed by ELISA for (b) eotaxin, (c) IL-5. (d) Periodic acid-Schiff score as a measure of airway mucus production. Each data point represents one mouse. (e) eosinophilia in BAL fluid and (f) TARC (CCL17). Data are representative of 3 independent experiments ($n = 5$ mice per group), with the exception of (d), which are from a single data set. ND, not detected.

Lung and blood neutrophils are not dysregulated following anti-IL-25 and anti-IL-17A administration

The pro-inflammatory functions of IL-17A, especially its ability to induce neutrophilia, are well documented, and intra-tracheal administration of IL-17A has been shown to increase the absolute number of neutrophils in the BAL [29]. Accumulation of neutrophils is a feature of both bronchial asthma and chronic obstructive pulmonary disease [30, 31] and is particularly associated with corticosteroid resistance [32], occupational asthma [33], and asthma in smokers [34]. To determine whether the elevated levels of IL-17A were inducing neutrophilia, and therefore potentially suppressing type-2 pathology, we undertook differential cell counts on blood and BAL cytopins. We observed no significant changes in the proportion of neutrophils in either blood (Fig. 3a) or BAL (Fig. 3b). These data suggest that the rising levels of IL-17A following anti-IL-25 monoclonal antibody treat-

ment do not simply induce neutrophils into the lung, and thus suppress the type-2 response independently of IL-25.

The effects of IL-25 and IL-17A on airway hyperresponsiveness and type-2 pathology are not due to changes in cell infiltration into the lung

The possibility existed that BAL cytokine production, following anti-IL-25 and/or anti-IL-17A treatment during lung allergy, was being regulated due to changes in lung cell infiltration and cell proliferation. However, on investigation lung cell infiltration was not affected by IL-25 and IL-17A levels (Fig. 4a). In addition, the number of cells recovered from the draining lymph nodes of OVA-sensitized and challenged animals was not significantly different between animals administered with anti-IL-25 and/or anti-IL-17 or control animals treated with isotype-matched antibodies (data not shown).

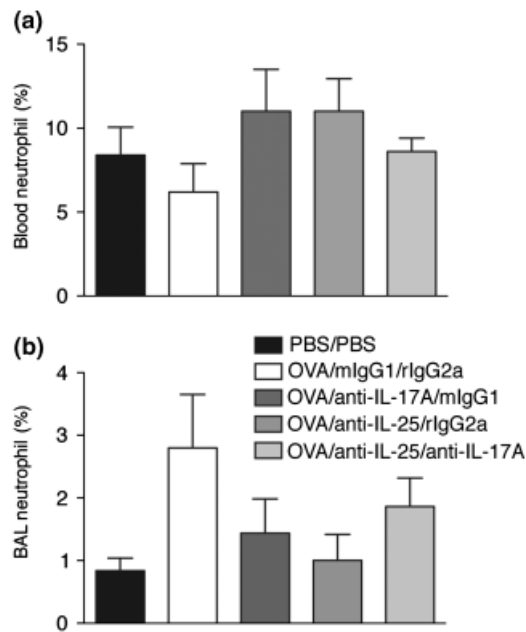


Fig. 3. No changes in neutrophilia following anti-IL-25 and anti-IL-17A treatment. The percentages of (a) blood and (b) bronchoalveolar lavage neutrophilia were assessed by differential cell count. Data are representative of three independent experiments ($n = 5$ per group).

Furthermore, no effect of anti-IL-25 or anti-IL-17 antibodies on proliferative capacity or cytokine production was observed after antigen restimulation of cells isolated from the draining lymph nodes (data not shown). Furthermore, intracellular flow cytometric analysis of cells isolated from disaggregated lung tissue (Figs 4b and c) showed that while the percentage of IL-13- and IL-17A-producing cells increased following OVA challenge, it was not affected by anti-IL-25 and anti-IL-17A treatment. However, investigation of intracellular IL-17A production in BAL cells showed that while the number of T cells producing IL-17A did not alter following antibody treatment (data not shown), the increase of a population of IL-17A-producing non-B/non-T ($CD3^-CD19^-$) cells did correlate with anti-IL-25 treatment as compared with isotype OVA-treated control mice (Fig. 4d).

Anti-IL-25 monoclonal antibody regulates IL-17A through IL-13 levels

Since IL-13 has been reported to counter-regulate IL-17A during EAE we wished to determine whether the regulation of IL-17A was caused directly by IL-25 or mediated

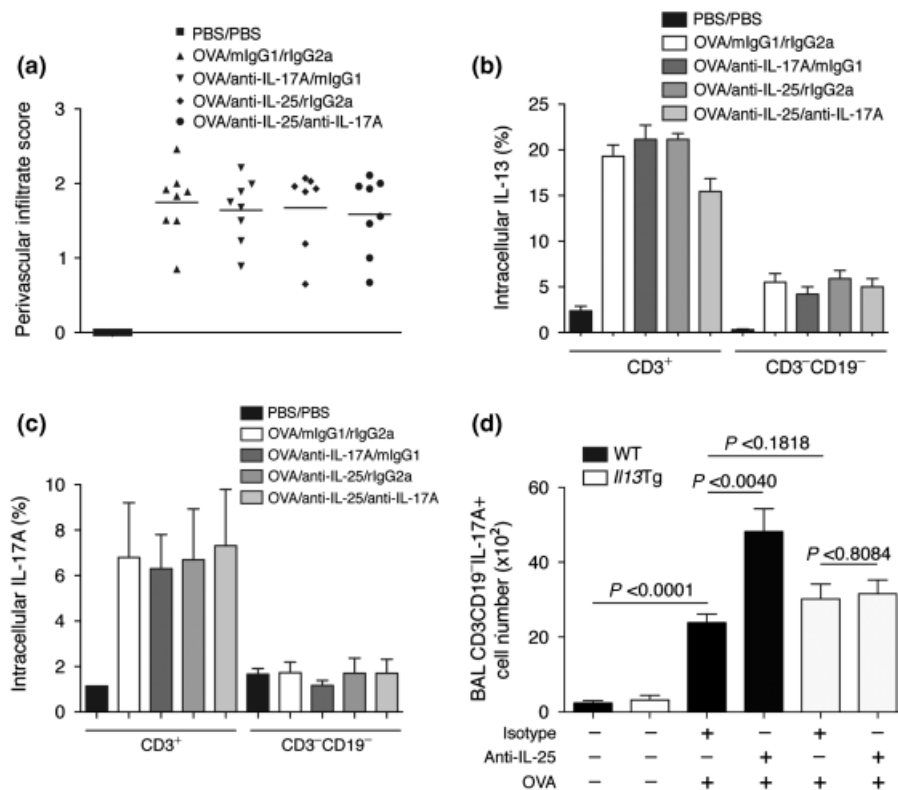


Fig. 4. IL-13 and IL-17A production by T cells and non-B/non-T cells as assessed by intracellular flow cytometry. (a) Perivascular cell infiltrate was assessed on paraffin-embedded histological sections stained with Giemsa. Data are representative of three independent experiments ($n = 5$ per group). Intracellular staining for IL-13 (b) and IL-17A (c) producing cells in disaggregated lung tissue. Data plots are representative of five mice per group. No statistical significance was found by Student *t*-test between any of the ovalbumin treatment groups. (d) Intracellular staining for IL-17A-producing cells in bronchoalveolar lavage. Data plots are representative of six mice per group.

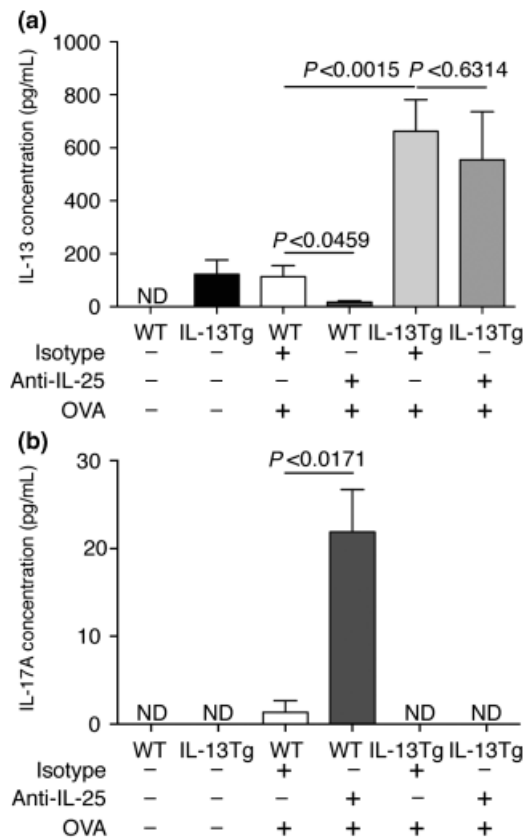


Fig. 5. Elevated IL-13 in *Il13Tg* mice suppresses IL-17A expression following anti-IL-25 treatment. (a) IL-13 levels in bronchoalveolar lavage (BAL). (b) IL-17A levels in BAL. Data are representative of 6 mice per group. ND, not detected.

through a downstream IL-13 effect. To test the role of IL-13 we made use of *Il13Tg* mice that express IL-13 from the *Il13* promoter in the presence of the human CD2 locus control region. These mice produce elevated levels of IL-13 even in the absence of antigen stimulation compared wild-type controls (Fig. 5a). Following OVA challenge *Il13Tg* mice express approximately eightfold higher levels of IL-13 than wild-type controls and in contrast to the wild-type animals anti-IL-25 treatment does not inhibit IL-13 production (Fig. 5a), meaning that we can assess the affect on IL-17A production when anti-IL-25 antibody is present but IL-13 levels are high. Notably, while anti-IL-25 administration resulted in the up-regulation of IL-17A in wild-type mice, anti-IL-25 did not result in elevated levels of IL-17A when IL-13 expression was maintained at elevated levels in the *Il13Tg* mice (Fig. 5b). Interestingly, we were also unable to detect an increase in IL-17A-producing non-B/non-T (CD3⁺CD19⁻) cells in the *Il13Tg* anti-IL-25 OVA-treated mice (Fig. 4d).

Discussion

We demonstrate a novel role for IL-25-induced IL-13 in suppressing the protective effects of IL-17A in allergic

lung inflammation. Neutralizing IL-25 in an experimental model of allergic asthma resulted in down-regulation of IL-13 levels and a reciprocal increase in the levels of IL-17A, correlating with prevention of AHR. When these elevated levels of IL-17A were neutralized by anti-IL-17A antibody treatment the levels of IL-13 recovered, as did eotaxin production and eosinophilia, and AHR returned to normal. In *Il13Tg* mice, that express elevated levels of IL-13 that cannot be inhibited by anti-IL-25 treatment, IL-17A is not up-regulated, indicating that IL-13 levels downstream of IL-25 are important for suppressing protective IL-17A production.

Our data also demonstrate that following neutralization of IL-25 a population of non-B/non-T IL-17A-producing cells arises in the BAL and correlate with protection from asthma. These cells do not appear in *Il13Tg* mice. Although we have been unable to identify these cells due to their extremely low number they may represent known IL-17A-producing lineages such as neutrophils or NK cells. A further possibility is that they may represent recently identified IL-17A-producing innate lymphoid cells [35, 36]. While we found no evidence for modulation of Th17 cells upon anti-IL-25 treatment it has recently been shown that Th17 cells may express functional IL-13 receptors and respond to IL-13 by down-regulating IL-17A expression [37]. It is possible that this type of regulation may affect the non-T/non-B IL-17A-producing cells that we observed in the BAL and it will be interesting to determine whether these cells express functional IL-13 receptors.

IL-13 is known to exacerbate allergic lung inflammation, and experiments using soluble IL-13R α 2-Fc fusion, or *Il13*^{-/-} mice, have been shown to ameliorate allergen-induced AHR and reduce mucus in the airways in a mouse model of asthma [3, 38]. Our initial observation of lower levels of IL-13 occurring following anti-IL-25 antibody treatment suggested a relatively simple model in which neutralization of IL-25 led to inhibition of AHR primarily through restricting local induction of IL-13 in the lung tissue and bronchoalveolar space, and consequently the downstream blocking of IL-13-induced effector functions such as mucus production and enhancing muscle contractility [39, 40]. However, our data now indicate that IL-25 regulation of IL-13 is also important for controlling IL-17A levels. Levels of IL-17A rise during treatment with anti-IL25 and this elevated IL-17A is essential for reducing AHR and pulmonary inflammation, through suppression of specific inflammatory mediators. It is also interesting to speculate that the balance of IL-25 and IL-17A, which can utilize the same receptor, IL-17RA, is perhaps critical to the production of IL-13 in the lung during allergic asthma. Anti-IL-17A treatment alone does not result in regulation of IL-13 production in our model, suggesting that only when IL-25 is neutralized can IL-17A out-compete IL-25 for their receptor and alter IL-13 production. Further experiments will be required to

determine how IL-17A production inhibits IL-13. To date our studies have used the model antigen OVA complexed with alum, where endotoxin has been removed, during the sensitization period. Further studies would be required to ascertain the significance of this finding using other complex antigens such as ragweed or house dust mite that have a greater capacity for activating toll like receptor pathways.

Although previous studies using mice deficient in IL-17A or IL-17A receptor reported that IL-17A was necessary for the development of AHR or pulmonary inflammation in an OVA-induced model of asthma [24, 41], a protective role for IL-17A has been suggested recently [24]. Schnyder-Candrian *et al.* [24] reported that although IL-17A is essential for the establishment of allergic asthma, administration of IL-17A to already sensitized mice could attenuate the allergic response [24], possibly by mediating TARC control of cell infiltration. Our data now show that the protective effects of IL-17A are likely to be dependent on the suppression of IL-25 and, consequently, IL-13. Although we failed to observe significant changes in TARC production in the BAL following anti-IL-25 and anti-IL-17A treatment, we found eotaxin and eosinophilia to be abrogated in parallel with decreases in IL-13 levels. These data are in keeping with previous reports in which IL-13, IL-4 and eotaxin have been demonstrated to play key roles in the tissue distribution of eosinophils [39, 42]. Independently of this others have reported the absence of an AHR response in mice genetically deficient in eosinophils [43], though the specific involvement of eosinophils in AHR remains contentious. Notably, we did not observe any changes in BAL eosinophilia or mucus production in the lung. This mirrors observations made by Ballantyne *et al.* [13] showing a disconnect between the effect of anti-IL-25 on inflammation and AHR at this time-point. Although other reports have shown IL-17A to induce neutrophilia we failed to observe significant changes in neutrophil infiltration, possibly due to the specific antigen used, temporal effects, or the context of an already committed type-2 response.

It is noteworthy that Kleinschek *et al.* [25] recently demonstrated that IL-13 could block *in vitro* differentiation of Th17 cells by inhibiting dendritic cell production of the Th17-promoting cytokines IL-1 β , IL-6, and IL-23 [25], and our data indicate that it is the regulation of IL-13 that acts as the switch between either a dominant IL-25 response or a dominant IL-17A directed response. This suggests that counterregulation of these cytokines is important in type-2-dependent allergic asthma in a similar fashion to these cytokines acting as a key determinant in the control of type-1 or 17-dependent EAE [25]. The findings also highlight the critical importance of maintaining the equilibrium of IL-25 and IL-17A in disease. This novel mechanistic observation, for the control of allergic AHR and the underlying type-2 directed pathology, is also potentially important for any therapeutic use of anti-IL-25 in treating asthma.

Elevated IL-17A has been reported to be associated with neutrophilic infiltrates in the lungs and increased asthma severity [44–46]. However these results are difficult to interpret due to the heterogeneity of asthma in humans, particularly as the role of neutrophils in asthma has not been defined. It is possible that the increased levels of IL-17A may be a consequence of severe inflammation and tissue damage rather than a cause. Here we demonstrate that rather than causing changes in neutrophil populations, the balance of IL-25, IL-13, and IL-17A is responsible for regulating type-2 responses during lung allergy.

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References

- 1 Grunig G, Warnock M, Wakil AE *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; **282**:2261–63.
- 2 Umetsu DT, McIntire JJ, Akbari O, Macaubas C, DeKruyff RH. Asthma: an epidemic of dysregulated immunity. *Nat Immunol* 2002; **3**:715–20.
- 3 Wills-Karp M, Luyimbazi J, Xu X *et al.* Interleukin-13: central mediator of allergic asthma. *Science* 1998; **282**:2258–61.
- 4 Fransson J, Teplyakov A, Raghunathan G *et al.* Human framework adaptation of a mouse anti-human IL-13 antibody. *J Mol Biol* 2010; **398**:214–31.
- 5 Singh D, Kane B, Molfino NA, Faggioni R, Roskos L, Woodcock A. A phase 1 study evaluating the pharmacokinetics, safety and tolerability of repeat dosing with a human IL-13 antibody (CAT-354) in subjects with asthma. *BMC Pulm Med* 2010; **10**:3–11.
- 6 Neill DR, Wong SH, Bellosi A *et al.* Nuocytes represent a new innate effector leukocyte that mediates type-2 immunity. *Nature* 2010; **464**:1367–70.
- 7 Fallon PG, Ballantyne SJ, Mangan NE *et al.* Identification of an interleukin (IL)-25-dependent cell population that provides IL-4, IL-5, and IL-13 at the onset of helminth expulsion. *J Exp Med* 2006; **203**:1105–16.
- 8 Owyang AM, Zaph C, Wilson EH *et al.* Interleukin 25 regulates type 2 cytokine-dependent immunity and limits chronic inflammation in the gastrointestinal tract. *J Exp Med* 2006; **203**:843–49.
- 9 Fort MM, Cheung J, Yen D *et al.* IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* 2001; **15**:985–95.
- 10 Hurst SD, Muchamuel T, Gorman DM *et al.* New IL-17 family members promote

- Th1 or Th2 responses in the lung: in vivo function of the novel cytokine IL-25. *J Immunol* 2002; **169**:443–53.
- 11 Kim MR, Manoukian R, Yeh R *et al.* Transgenic overexpression of human IL-17E results in eosinophilia, B-lymphocyte hyperplasia, and altered antibody production. *Blood* 2002; **100**:2330–40.
 - 12 Pan G, French D, Mao W *et al.* Forced expression of murine IL-17E induces growth retardation, jaundice, a Th2-biased response, and multiorgan inflammation in mice. *J Immunol* 2001; **167**: 6559–67.
 - 13 Ballantyne SJ, Barlow JL, Jolin HE *et al.* Blocking IL-25 prevents airway hyperresponsiveness in allergic asthma. *J Allergy Clin Immunol* 2007; **120**:1324–31.
 - 14 Wang YH, Angkasekwinai P, Lu N *et al.* IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007; **204**:1837–47.
 - 15 Angkasekwinai P, Park H, Wang YH *et al.* Interleukin 25 promotes the initiation of proallergic type 2 responses. *J Exp Med* 2007; **204**:1509–17.
 - 16 Ikeda K, Nakajima H, Suzuki K *et al.* Mast cells produce interleukin-25 upon Fc epsilon RI-mediated activation. *Blood* 2003; **101**:3594–96.
 - 17 Kang CM, Jang AS, Ahn MH *et al.* Interleukin-25 and interleukin-13 production by alveolar macrophages in response to particles. *Am J Respir Cell Mol Biol* 2005; **33**:290–96.
 - 18 Letuve S, Lajoie-Kadoch S, Audusseau S *et al.* IL-17E upregulates the expression of proinflammatory cytokines in lung fibroblasts. *J Allergy Clin Immunol* 2006; **117**:590–96.
 - 19 Lajoie-Kadoch S, Joubert P, Letuve S *et al.* TNF-alpha and IFN-gamma inversely modulate expression of the IL-17E receptor in airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2006; **290**:L1238–46.
 - 20 Weaver CT, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; **25**:821–52.
 - 21 Happel KI, Dubin PJ, Zheng M *et al.* Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J Exp Med* 2005; **202**:761–69.
 - 22 Chung DR, Kasper DL, Panzo RJ *et al.* CD4+ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. *J Immunol* 2003; **170**:1958–63.
 - 23 Huang W, Na L, Fidel PL, Schwarzenberger P. Requirement of interleukin-17A for systemic anti-*Candida albicans* host defense in mice. *J Infect Dis* 2004; **190**:624–31.
 - 24 Schnyder-Candrian S, Togbe D, Couillin I *et al.* Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med* 2006; **203**:2715–25.
 - 25 Kleinschek MA, Owyang AM, Joyce-Shaikh B *et al.* IL-25 regulates Th17 function in autoimmune inflammation. *J Exp Med* 2007; **204**:161–70.
 - 26 Rickel EA, Siegel LA, Yoon BR *et al.* Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 2008; **181**:4299–310.
 - 27 Emson CL, Bell SE, Jones A, Wisden W, McKenzie AN. Interleukin (IL)-4-independent induction of immunoglobulin (Ig)E, and perturbation of T cell development in transgenic mice expressing IL-13. *J Exp Med* 1998; **188**:399–404.
 - 28 Tong J, Bandulwala HS, Clay BS *et al.* Fas-positive T cells regulate the resolution of airway inflammation in a murine model of asthma. *J Exp Med* 2006; **203**:1173–84.
 - 29 Laan M, Linden A. IL-17 as a potential target for modulating airway neutrophilia. *Curr Pharm Des* 2002; **8**:1855–61.
 - 30 O'Donnell RA, Peebles C, Ward JA *et al.* Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD. *Thorax* 1994; **59**:837–42.
 - 31 Ordonez CL, Shaughnessy TE, Matthay MA, Fahy JV. Increased neutrophil numbers and IL-8 levels in airway secretions in acute severe asthma: clinical and biologic significance. *Am J Respir Crit Care Med* 2000; **161**:1185–90.
 - 32 Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; **57**:875–79.
 - 33 Anees W, Huggins V, Pavord ID, Robertson A, Burge PS. Occupational asthma due to low molecular weight agents: eosinophilic and non-eosinophilic variants. *Thorax* 2002; **57**:231–36.
 - 34 Chalmers GW, MacLeod KJ, Thomson L, Little SA, McSharry C, Thomson NC. Smoking and airway inflammation in patients with mild asthma. *Chest* 2001; **120**:1917–22.
 - 35 Buonocore S, Ahern PP, Uhlig HH *et al.* Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature* 2010; **464**:1371–75.
 - 36 Sawa S, Cherrier M, Lochner M *et al.* Lineage relationship analysis of ROR-gammat+innate lymphoid cells. *Science* 2010; **330**:665–69.
 - 37 Newcomb DC, Zhou W, Moore ML *et al.* functional IL-13 receptor is expressed on polarized murine CD4+Th17 cells and IL-13 signaling attenuates Th17 cytokine production. *J Immunol* 2009; **182**:5317–21.
 - 38 Walter DM, McIntire JJ, Berry G *et al.* Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J Immunol* 2001; **167**:4668–75.
 - 39 Fallon PG, Jolin HE, Smith P *et al.* IL-4 induces characteristic Th2 responses even in the combined absence of IL-5, IL-9, and IL-13. *Immunity* 2002; **17**:7–17.
 - 40 Tliba O, Deshpande D, Chen H *et al.* IL-13 enhances agonist-evoked calcium signals and contractile responses in airway smooth muscle. *Br J Pharmacol* 2003; **140**:1159–62.
 - 41 Nakae S, Komiyama Y, Nambu A *et al.* Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 2002; **17**:375–87.
 - 42 Yang M, Hogan SP, Henry PJ *et al.* Interleukin-13 mediates airways hyperreactivity through the IL-4 receptor-alpha chain and STAT-6 independently of IL-5 and eotaxin. *Am J Respir Cell Mol Biol* 2001; **25**:522–30.
 - 43 Lee JJ, Dimina D, Macias MP *et al.* Defining a link with asthma in mice congenitally deficient in eosinophils. *Science* 2004; **305**:1773–76.
 - 44 Barczyk A, Pierzchala W, Sozanska E. Interleukin-17 in sputum correlates with airway hyperresponsiveness to methacholine. *Respir Med* 2003; **97**:726–33.
 - 45 Hashimoto T, Akiyama K, Kobayashi N, Mori A. Comparison of IL-17 production by helper T cells among atopic and non-atopic asthmatics and control subjects. *Int Arch Allergy Immunol* 2005; **137** (Suppl. 1):51–54.
 - 46 Molet S, Hamid Q, Davoine F *et al.* IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J Allergy Clin Immunol* 2001; **108**:430–38.