

Direct comparison of the dynamics of IL-25- and 'allergen'-induced airways inflammation, remodelling and hypersensitivity in a murine asthma model

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Clinical & Experimental Allergy

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Cite this as: X. J. Yao, K. W. Huang, Y. Li, Q. Zhang, J. J. Wang, W. Wang, J. Liu, Z. Lv, Y. Q. An, Y. Z. Ding, C. J. Corrigan, W. Wang, Y. C. Sun and S. Ying, *Clinical & Experimental Allergy*, 2014 (44) 765–777.

Summary

Background Interleukin-25 has been implicated in the pathogenesis of asthma from studies on human asthmatics and in murine asthma models.

Objectives In this study, we hypothesized that chronic exposure of the airways to IL-25 alone is able to induce pathogenic changes observed in animal models of asthma.

Methods We performed a detailed comparison of the dynamics of development of cellular infiltration, cytokine expression and airways remodelling and hyperresponsiveness in mice sensitized and challenged with OVA, a classical model of allergic asthma and those exposed to IL-25 alone.

Results Intranasal challenge of BALB/c mice with IL-25 alone induced a delayed (compared with OVA-challenge), predominantly eosinophilic and lymphocytic infiltration into the airways lumen, along with increased production of Th2-type cytokines, chemokines and collagen, secretion of epithelial mucus, goblet cell hyperplasia, deposition of subepithelial collagen, airways smooth muscle cell hyperplasia and angiogenesis. Correspondingly, IL-25 as well as OVA challenge both induced airways hyperresponsiveness and increased lung tissue damping. In contrast, IL-25 exposure did not increase IgE or IgG₁ production.

Conclusions and Clinical Relevance The data suggest that chronic airways exposure to IL-25 alone is sufficient to induce allergen- and IgE-independent, asthma-like airways inflammation, remodelling and hyperresponsiveness in mice. Thus, IL-25 is a key molecular target in asthma, irrespective of the coexistence of IgE-dependent mechanisms.

Keywords asthma, IL-25, IL-25 receptor, murine model, pathogenesis, remodelling

Submitted 26 September 2013; revised 24 January 2014; accepted 15 February 2014

Introduction

Asthma is one of the most common chronic diseases in industrialized countries, and its frequency is predicted to increase world-wide in the next decade. Airways hyperresponsiveness (AHR), inflammation, remodelling and reversible obstruction are thought to be central features of asthma. Although many patients are satisfactorily controlled in terms of symptoms and airways

inflammation by current medications, there are few drugs known effectively to control airways remodelling, a collective term used to describe structural changes seen in the airways of asthmatics which comprise of epithelial hypertrophy and metaplasia, goblet cell hyperplasia, deposition of subepithelial fibrous and other extracellular matrix proteins, myofibroblast hyperplasia, airways smooth muscle (ASM) cell hyperplasia and angiogenesis.

Interleukin-25 (IL-17E) is a member of the IL-17 cytokine family, which is exceptional in promoting

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Th2-type immune responses. In mice, exogenous administration of IL-25 [1, 2] or transgenic expression [3, 4] induces asthma-like features, with airways hyper-responsiveness, eosinophil infiltration and airways changes recapitulating those seen in human asthma, possibly mediated at least partly by enrichment of 'nuocytes' expressing IL-5/IL-13 [5].

Conversely, anti-IL-25 antibody reduces infiltration of allergen-specific Th2 T cells and eosinophils [6] and inhibits some processes associated with airways remodelling in human asthma, such as collagen lay down, in the airways of animals respectively challenged intranasally with proteins such as ovalbumin or human allergens, which induce an IgE response which causes an inflammatory reaction in the airways initially dependent on type I hypersensitivity [7, 8].

We have previously demonstrated increased expression of IL-25 and its receptor in the human asthmatic bronchial mucosa at baseline and following allergen challenge [9–11], and that binding of IL-25 to IL-25R/IL-17RB, but not IL-17RA enhances human lung vascular endothelial cell proliferation and angiogenesis *in vitro* through up-regulation of bFGF, VEGF and VEGF receptors [10, 12]. In addition to this evidence, some studies have observed the expression of IL-25R on bronchial epithelial cells, lung fibroblasts and smooth muscle cells [8, 13, 14], suggesting that IL-25 may also contribute to airways remodelling by acting on its receptor expressed by these structural cells.

In this study, we hypothesized that chronic exposure of the airways to IL-25 alone is able to induce inflammatory and remodelling changes reminiscent of human asthma as well as airways hyperresponsiveness by mechanisms independent of and distinct from artificially induced type I hypersensitivity responses. To address this hypothesis, we compared, in parallel experiments, the effects of serial challenge of groups of mice either with IL-25 alone or with ovalbumin (OVA) (the animals challenged with OVA required prior intraperitoneal sensitization to induce an IgE response). We compared, in each cohort of animals, systemic IgE production, airways inflammation, lung function and remodelling changes.

Methods

Animals

Female BALB/c mice (8–10 week old) were purchased from Vital River Laboratories (Beijing, China) and maintained in a pathogen-free mouse facility located in the Department of Laboratory Animal Sciences, Capital Medical University, Beijing, China. All experiments were approved by the Institutional Animal Care and Use Committee (IACUC).

Allergen- and IL-25-induced airways inflammation and remodelling

To compare IL-25-induced airways changes with those in 'standard' models of allergen-induced asthma, mice were assigned to 3 groups, including a group serially challenged with 50 µg/dose of OVA [15], a group challenged with saline as a negative control and a group serially challenged with IL-25 [16]. Briefly, mice challenged with OVA were first sensitized by intraperitoneal injection of OVA (Sigma-Aldrich, Beijing, China, 100 µg emulsified in Al(OH)₃/dose) on days 0 and 12. On days 18 to 23, these mice were further challenged daily intranasally with 50 µg of OVA (OVA50) in 50 µL saline/dose. Mice in the IL-25-challenged group were not sensitized or exposed to OVA, but subjected to daily nasal challenge from days 18 to 23 with recombinant mouse IL-25 (mIL-25, R&D Systems, 2 µg in 50 µL saline) [16]. Subsequently, the mice were further challenged intranasally either with OVA or with IL-25 every 2 days for a further 30 days. Some mice in each group were observed for a further 17 days after stopping the challenges. Control mice were intraperitoneally injected with same amount of Al(OH)₃ then nasally challenged with saline at time-points corresponding to those in the other groups (Fig. 1a).

Invasive lung function measurements

Measurements were taken on days 20, 24, 36, 48, 55 and 70, 24 hrs after the most recent intranasal challenge. A baseline was established by measurement at day 0. Lung function was measured using the FlexiVent system (Sciencelab Inc., Montreal, Canada) as described previously [17, 18]. Briefly, mice were anaesthetized with intraperitoneal injections of pentobarbital sodium at a dose of 80 mg/kg body weight, tracheostomized and ventilated, and pulmonary mechanics measured at a rate of at 150 breaths/min with a tidal volume of 8 mL/kg and positive end expiratory pressure of 2 cmH₂O. Methacholine was delivered via a nebulizer; after each dose, the response was measured by applying 2-s perturbations at 10-s intervals for a total of 3 min. At each time-point, dose-response curves were determined for each of three parameters of lung function as described below.

Bronchoalveolar lavage fluid collection and characterization

Bronchoalveolar lavage fluid (BALF) was collected from mice immediately following euthanasia as previously described [17, 18]. Supernatants were stored at –80°C until used. Cytospins were prepared from the cell pellets, and differential cell counts performed with haematoxylin/eosin (H&E) staining.

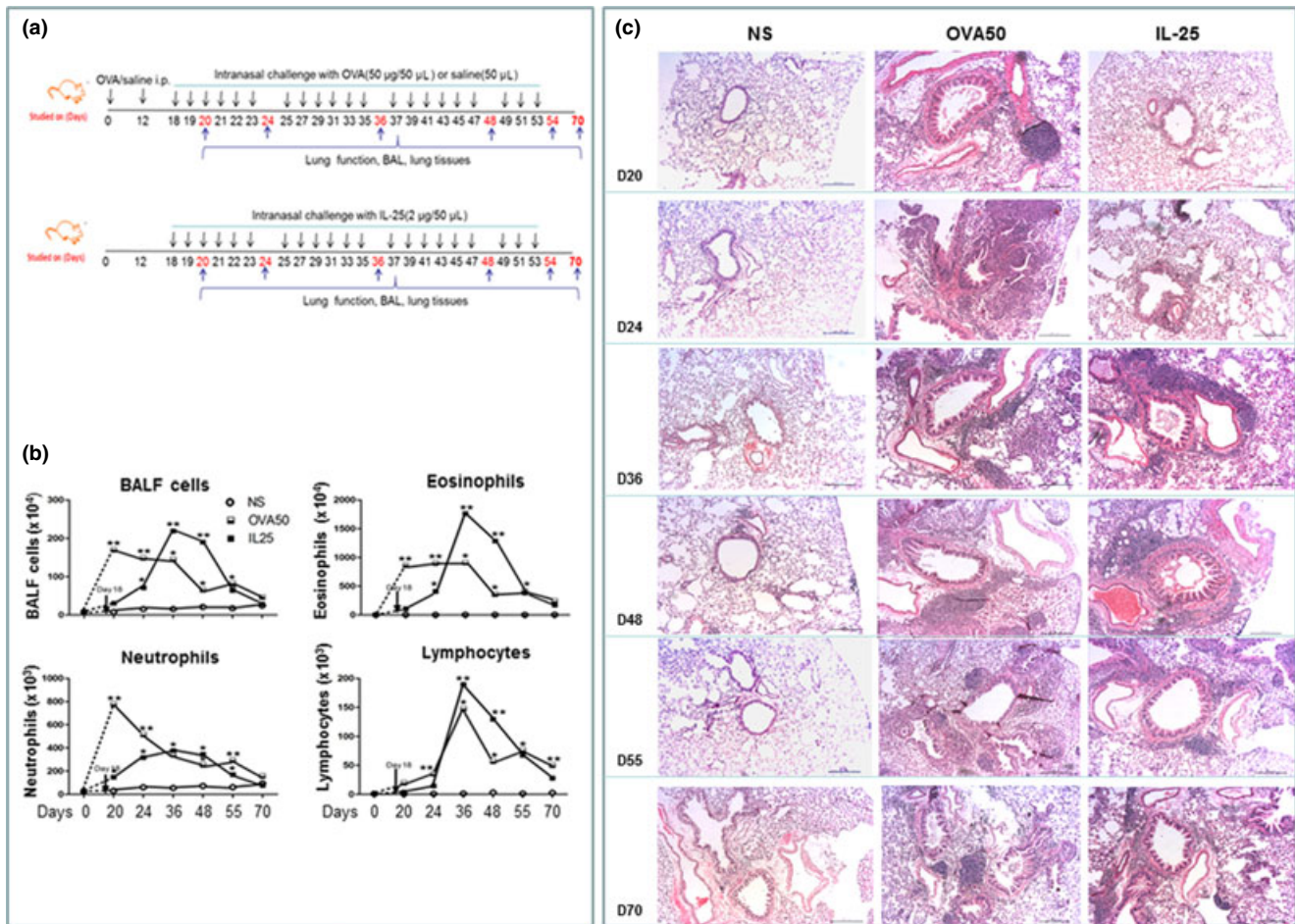


Fig. 1. (a) Schedule of murine challenge. (b) Total cells, eosinophils, neutrophils and lymphocytes in BAL fluid of OVA-, IL-25- and saline (NS)-challenged mice at various time-points. Data were collected from three independent experiments and are expressed as mean changes ($n = 5$ in each group at each time-point). ** $P < 0.05$ vs. IL-25/or OVA and NS-challenged groups. * $P < 0.05$ vs. control group. (c) Representative photos of H&E-stained lung sections (original magnification $\times 10$).

Lung histology

Left lung lobes were inflated and fixed with 10% formalin for 24 h. Paraffin-embedded sections (5 µm) were stained with H&E to evaluate general morphology and inflammation, and with periodic acid-Schiff (PAS) (Beijing Yi Li Fine Chemistry Co Ltd, Beijing, China) to visualize goblet cells and evaluate airway mucus expression. The PAS staining was scored for the degree of goblet cell hyperplasia according to a previous report [19]. All scoring was performed by two independent observers ignorant of the origin of the specimens.

A Masson's trichrome kit (Nanjing Mindit Biochemistry Co Ltd, Nanjing, China) was used to visualize and measure airways fibrosis in lung tissue sections [15, 16]. Because eosinophilia is a major feature of asthmatic inflammation, and eosinophils are not easily distinguished from other inflammatory cells by routine H&E staining, additional Congo red (Beijing Solarbio Technology Co Ltd, Beijing, China) staining was used to

identify eosinophils in lung tissue sections [20]. Microscopy-based multicolour tissue cytometry (MMTC) was employed further to quantify Congo red-positive eosinophils using a HistoQuest software package (TissueGnostics GmbH, Vienna, Austria) [21].

Lung immunohistochemistry

Immunohistochemistry was used to detect smooth muscle and vascular endothelial cells. The primary monoclonal antibodies against mouse α -smooth muscle actin (1 : 200) and CD31 (endothelial cells) (1 : 50) were purchased from Abcam (Hong Kong, China). The peroxidase antiperoxidase (PAP) technique was used as previously described [10–12]. Positively staining cells were detected with the glucose oxidase–DAB–nickel method [22]. The slides were analysed by independent observers ignorant of their origin on a Leica DM6000B microscope (Leica, Wetzlar, Germany) connected with a Leica Application Suite, version 3.6. The data were

expressed as the total numbers of CD31⁺ vascular endothelial cells per unit area of entire lung sections. The thickness of the smooth muscle layer in larger- and middle-sized airways was measured as previously described [8].

Preparation of lung tissue homogenates, quantification of total collagen

Right lung tissue (100 mg) was homogenized in 2 mL PBS containing 1% Triton X-100 and a protease inhibitor cocktail tablet (Roche Diagnostics GmbH, Mannheim, Germany). Debris was removed by centrifugation, and the supernatant collected for measurement of total collagen, cytokines and chemokines. Collagen content was measured and normalized for tissue weight using a biochemical assay according to the manufacturer's instructions (Sircol collagen assay, Biocolor, Belfast, UK) [15].

Analysis of cytokines and chemokines

A commercial BDTM[™] Cytometric bead array (CBA) platform (BD Biosciences Pharmingen, San Diego, CA,

USA) was used to determine the concentrations of IFN- γ , IL-4 [23] and the murine IL-8 homologue (CXCL1/KC) in the supernatants of lung tissue homogenates [24]. The concentrations of IL-5, IL-13, IL-25, IL-33, TSLP (thymic stromal lymphopoietin) and eotaxin were measured with commercial ELISA kits (eBioscience, San Diego, CA, USA). Serum total IgE and IgG₁ antibody concentrations were measured by ELISA (eBioscience) as previously described [25]. Serum samples collected on day 0 were used as controls.

Statistical analysis

The results were analysed using InStat 2.01 software (GraphPad, San Diego, CA, USA). Differences between experimental groups were analysed using two-way ANOVA to establish significant variation with time within groups followed by the Student's unpaired *t*-test for between-group comparisons at specific time-points. Data are presented as the mean $\pm$ SEM. For all tests, *P*-values less than 0.05 were considered significant.

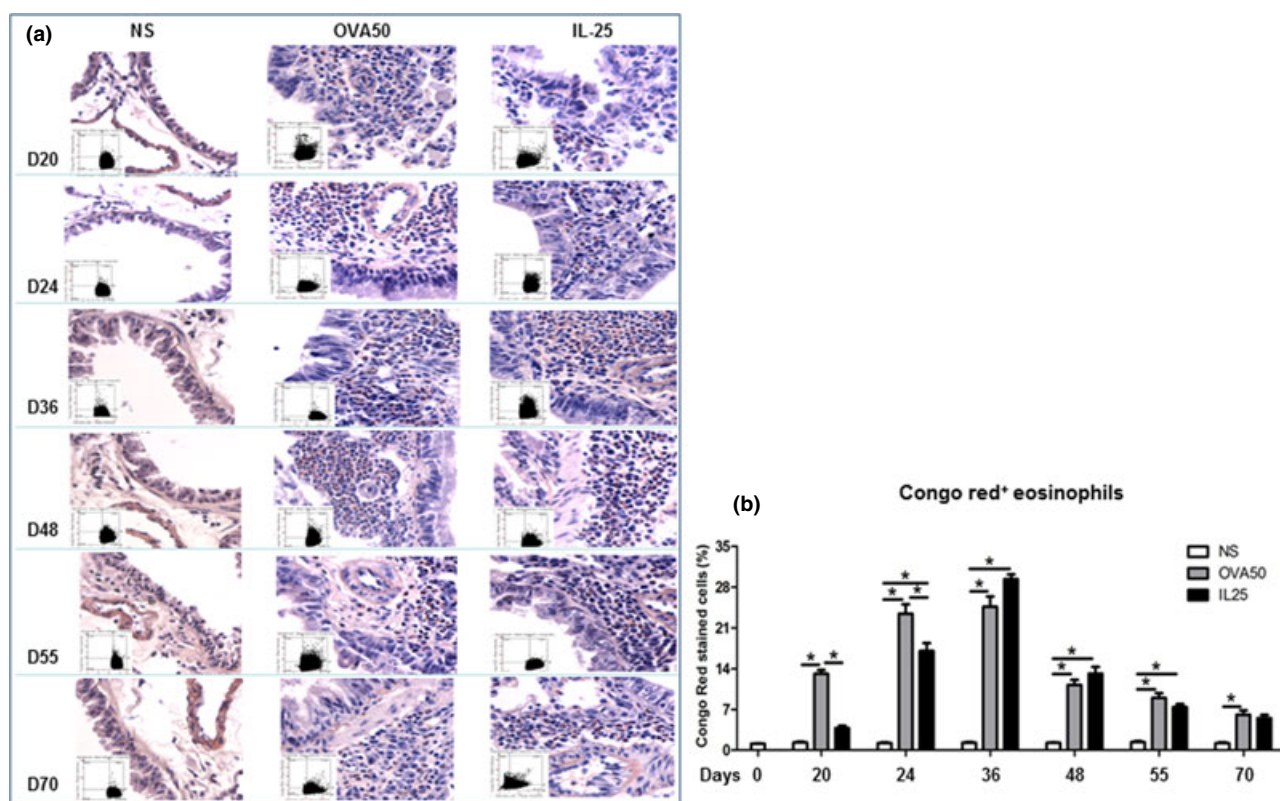


Fig. 2. (a) Representative photographs of Congo red⁺ eosinophils in lung sections. Representative images obtained by microscopy-based multicolour tissue cytometry (MMTC) are shown in the left corners of each individual photographs. (b) Quantitative MMTC analysis. The data were collected from three independent experiments and are expressed as mean $\pm$ SEM percentages of Congo red-stained total nucleated cells ($n = 5$ in each group at each time-point). * $P < 0.05$.

Results

IL-25-induced inflammatory cellular infiltration of the lung tissues and airways

Interleukin-25 challenge of naïve animals and OVA challenge of previously sensitized animals both caused florid infiltration of inflammatory cells into the airways and peribronchial and perivascular regions of the lung parenchyma (Fig. 1). Cells infiltrating into the airways lumen included a predominance of eosinophils accompanied by neutrophils and lymphocytes (Fig. 1b). Cellular infiltration was sustained during the entire period of challenge. Interestingly, the peak of infiltration of eosinophils and neutrophils into the airways was relatively delayed (peak day 36) with IL-25 as compared with OVA challenge (Fig. 1b), whereas peak lymphocyte infiltration was observed at day 36 in both groups (Fig. 1b). H&E staining of the airways and lung parenchyma showed that, compared with saline-challenged control mice, there were extensive mucosal folding and mucous hypertrophy with peribronchiolar and perivascular inflammation and mucus plug formation in

both the OVA- and IL-25-challenged mice, particularly after day 24 (Fig. 1c).

IL-25 induced lung eosinophilia

Lung sections stained with Congo red showed that along with eosinophil infiltration of the airways, IL-25 challenge induced a lung parenchymal and submucosal peribronchial and perivascular eosinophil infiltrate which persisted beyond day 55 (Fig. 2a). Microscopy-based multicolour tissue cytometry (MMTC) showed that the peak of IL-25-induced lung eosinophilia was at day 36, while the peak of OVA-induced lung eosinophilia was slightly earlier (Fig. 2b).

IL-25 induced airways goblet cell hyperplasia

Interleukin-25 inhalation challenge induced florid mucous hyperplasia in the bronchial epithelium as shown by PAS staining (Fig. 3a). This persisted from day 36 until the end of the study (day 70) (Fig. 3b). OVA-challenged animals developed maximal mucous hyperplasia by day 24, again somewhat earlier than the IL-25-challenged animals (Fig. 3b).

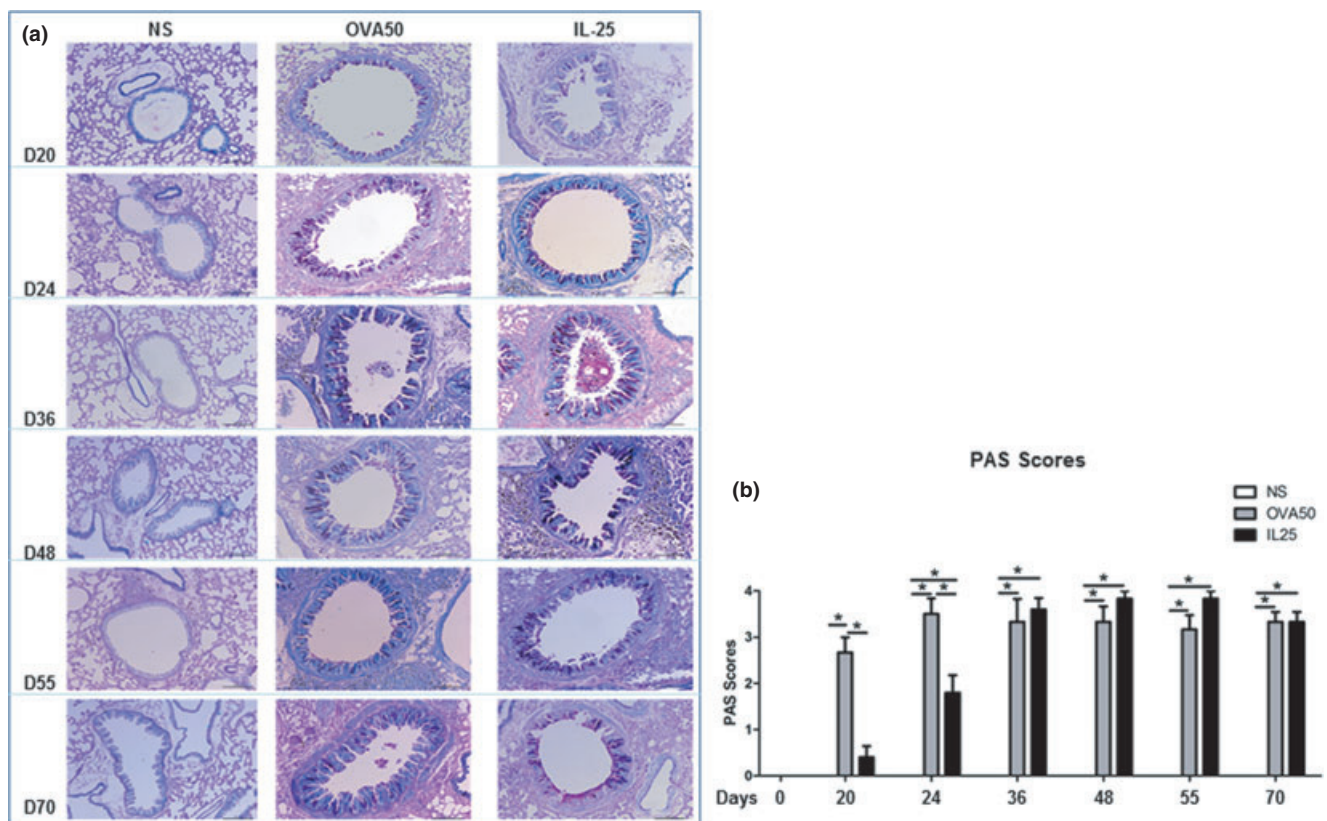


Fig. 3. (a) Representative photographs of periodic acid-Schiff (PAS) stained lung sections from saline (NS)-, OVA- and IL-25-challenged mice at various time-points as indicated (original magnification $\times 20$). (b) Mucus score based on PAS staining as described in Materials and Methods. Data were collected from three independent experiments and are expressed as mean $\pm$ SEM ($n = 5$ in each group at each time-point). $*P < 0.05$.

IL-25 increased lung matrix protein deposition

To determine whether IL-25 inhalation challenge induced matrix protein deposition in the lung of challenged animals, sections were stained with Masson's trichrome blue (MSB) to assess collagen deposition (Fig. 4a). No extensive collagen deposition, around airways or vasculature, was noted in the saline-challenged animals from days 20 to 70. Following intranasal inhalational challenge, animals exposed to IL-25 showed extensive peribronchial collagen deposition visually comparable to that induced by OVA and persisting until day 70 (Fig. 4a). To assess the extent of pulmonary matrix deposition quantitatively, total collagen in lung homogenates was measured using a commercial biological assay. IL-25 challenge induced significant increase in collagen compared with saline-challenged animals at day 48 and beyond (Fig. 4b). OVA-challenged animals showed similar increase in lung collagen.

IL-25 induced airways smooth muscle hypertrophy

Interleukin-25 challenge also induced hypertrophy of airways smooth muscle, which was significant at 48 days

and later time-points (Fig. 5a). Quantitative analysis of α -smooth muscle actin immunostaining verified this visual impression. The extent and time course of changes were comparable to those induced by OVA challenge (Fig. 5b).

IL-25 induced increased submucosal vascularity

Immunoanalysis showed that IL-25 inhalation challenge of the mice induced significantly increased numbers of CD31 immunoreactive blood vessels, both in the parenchyma and the submucosa of airways which was apparent from day 36 onwards (Figs 6a and b). Interestingly, the numbers of vessels declined rapidly after cessation of IL-25 exposure such that they were no longer significantly increased by day 70. OVA challenge induced similar changes, but these were significantly less marked than those induced by IL-25 with this particular regimen (Figs 6a and b).

IL-25 induced increased cytokine and chemokine expression in the lung parenchyma

Airways inflammation in asthma is postulated to occur partly through local release of cytokines and

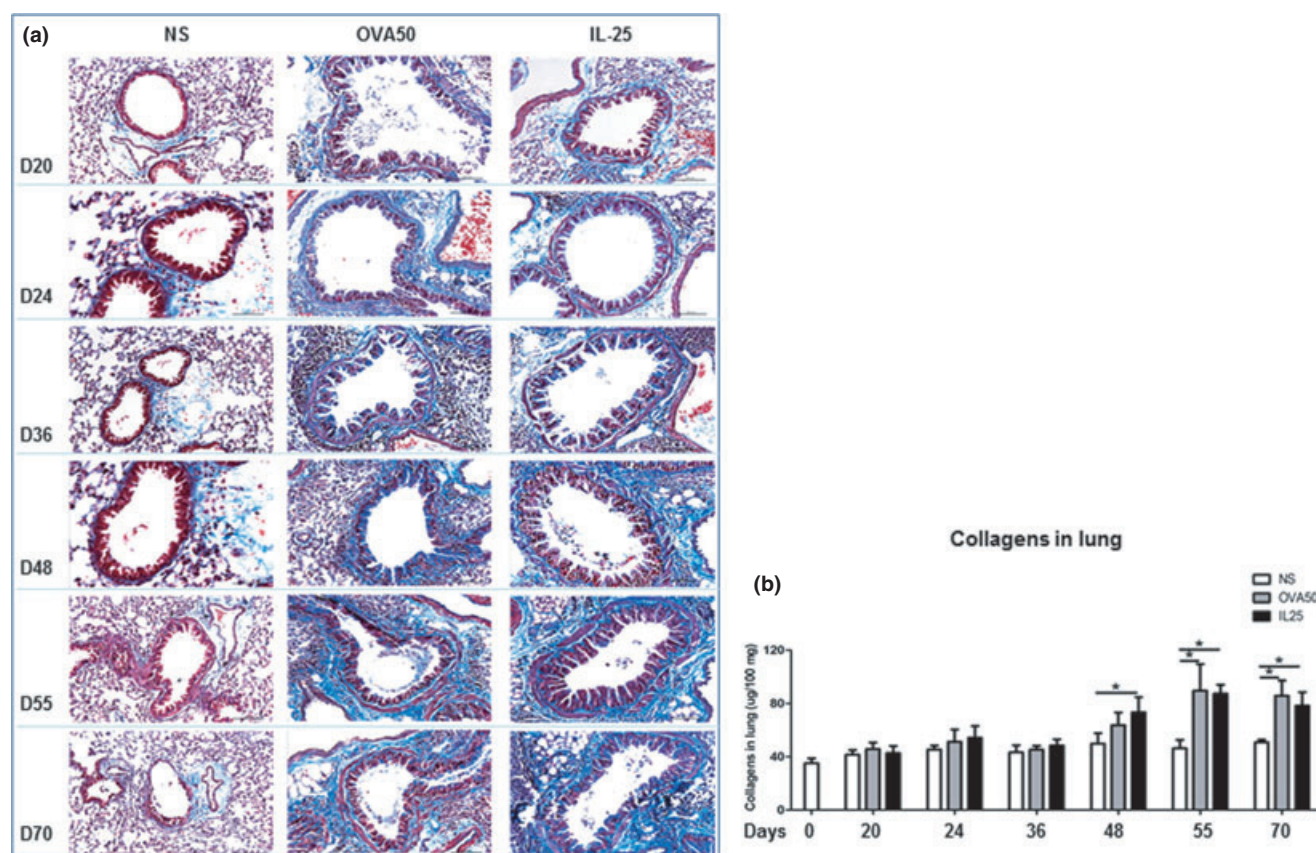


Fig. 4. (a) Representative photographs of Masson's trichrome-blue-stained lung sections from saline (NS)-, OVA- and IL-25-challenged mice at various time-points as indicated (original magnification $\times 20$). (b) Total lung collagen ($\mu\text{g}/100$ mg of total protein). Data were collected from three independent experiments and are expressed as mean $\pm$ SEM ($n = 5$ in each group at each time-point). * $P < 0.05$.

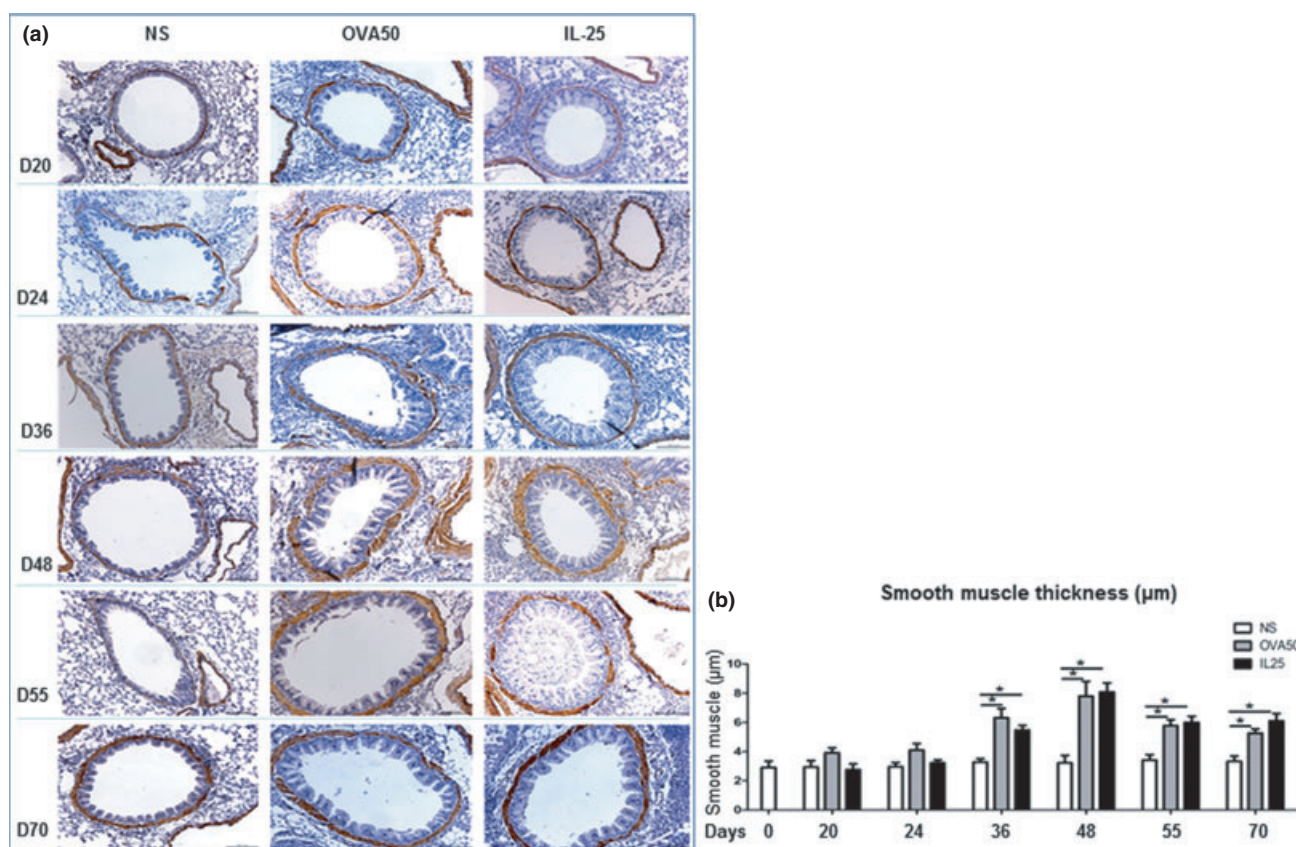


Fig. 5. (a) Representative photographs of α -smooth muscle actin immunoreactivity in lung sections from saline (NS)-, OVA- and IL-25-challenged mice at various time-points as indicated (original magnification $\times 20$). (b) Quantitative analysis of thickness of α -smooth muscle actin-positive muscle. Data were collected from three independent experiments and are expressed as mean $\pm$ SEM ($n = 5$ in each group at each time-point). * $P < 0.05$.

chemokines by immune and structural cells. Consequently, we measured concentrations of the Th1 cytokine IFN- γ , the Th2 cytokines IL-4, IL-5 and IL-13 and the chemokines eotaxin and KC (CXCL-1, a homologue of human IL-8) in supernatants of lung tissue homogenates from the mice challenged using our regimen. Cytometric bead array and ELISA data showed that OVA challenge induced increased expression of typical Th2, but not Th1 signature cytokines (Fig. 7). In contrast, IL-25 challenge induced increased production of IL-5 and IL-13, but not IFN- γ or IL-4 (Fig. 7).

Interleukin-25 challenge also increased production of the chemokine eotaxin, which was evident by day 24, persisted up to day 48 then slightly declined by day 55 (Fig. 7). In contrast, OVA-induced eotaxin production peaked relatively later at day 48 (Fig. 7). IL-25 also induced production of the chemokine KC (IL-8) with a similar time course to that produced by OVA challenge except that its production declined rather earlier to baseline by day 55 (Fig. 7).

Finally, IL-25 challenge was able to induce production of IL-33 and TSLP (Fig. 7), two major promoters of Th2-

mediated responses. Increased production of both mediators was apparent from day 20 until the end of the experiment. Again, OVA challenge similarly induced increased production of IL-33 and TSLP (Fig. 7).

IL-25 challenge did not increase IgE or IgG₁ production

Sensitisation followed by intranasal challenge of mice with OVA induced significant increases in concentrations of total serum IgE (Fig. 7) and IgG₁ (the murine equivalent of human IgG₄), which peaked at day 20 then gradually declined to baseline (IgE by day 55 and IgG₁ by day 48). In stark contrast to OVA challenge, IL-25 challenge did not significantly alter concentrations of total serum IgE or IgG₁ at any time-point throughout the duration of the experiment.

IL-25 challenge induced AHR and increased tissue damping

Intranasal challenge of mice with IL-25 alone induced AHR to methacholine, which was comparable to that

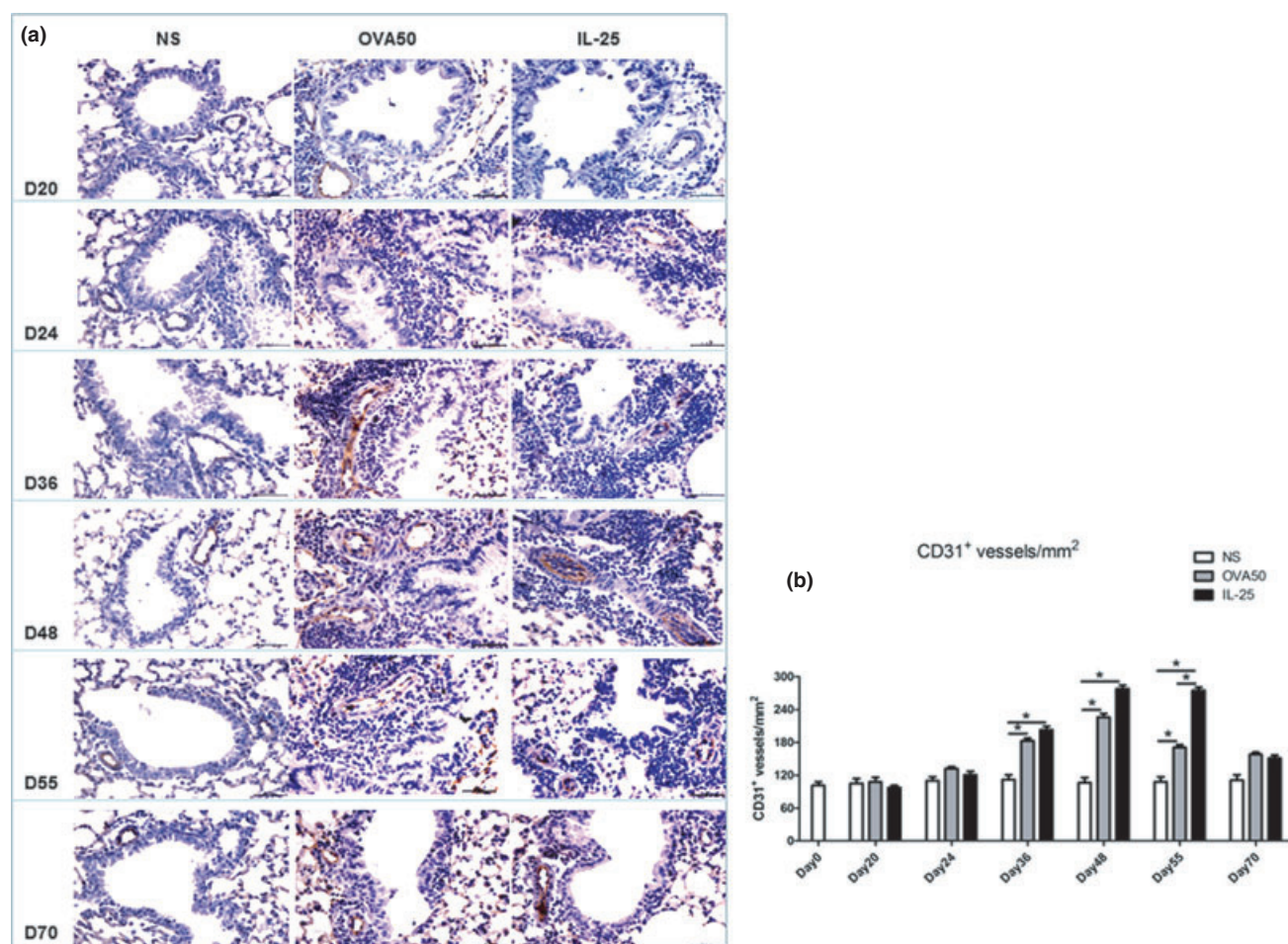


Fig. 6. (a) Representative photographs of CD31 immunoreactive blood vessels in lung sections from saline (NS)-, OVA- and IL-25-challenged mice at various time-points as indicated (original magnification $\times 40$). (b) CD31⁺ blood vessels per unit area. Data were collected from three independent experiments and are expressed as mean $\pm$ SEM ($n = 5$ in each group at each time-point). * $P < 0.05$.

observed in OVA-challenged animals and persisted up to day 48. As the timing of the successive challenges progressed, increasingly higher concentrations of methacholine were required to demonstrate AHR, possibly a result of airways remodelling (Fig. 8a). In support of this, tissue damping (G), a parameter which is closely related to tissue resistance and reflects the dissipative properties of the lung tissues, increased progressively with challenge (Fig. 8b). By day 70, 17 days after the final challenge, AHR to methacholine was similar in the IL-25-, OVA- and saline-challenged animals, while tissue damping (G) remained significantly increased in the IL-25 and OVA-challenged mice (Fig. 8b). Along with tissue damping (G), tissue elastance (H) also increased progressively up to day 55 (data not shown).

Discussion

Previous studies have addressed facets of the role of IL-25 in inducing changes associated with human

asthma in the airways of animals by direct application to the airways [2, 16], blockade or genetic knock-down of IL-25 during repeated antigen challenge designed to induce an IgE response [7, 8, 26] or over expression of IL-25 in airways epithelial cells [6]. The uniqueness and novelty of the present study lies in our side-by-side comparison of the direct effects of IL-25 with those induced in the course of 'allergic' changes in the airways dependent on IgE sensitization. This comparison was interesting because it highlighted marked differences in the dynamics of inflammatory cellular infiltration, particularly those of neutrophils and eosinophils which peaked much later following IL-25 compared with OVA challenge at day 36, corresponding to the peak of both IL-25- and OVA-induced lymphocyte infiltration. One potential explanation for this is a lack of acute, IgE-mediated mast cell degranulation following IL-25 challenge, which in conventional allergen challenge models is associated with rapid influx of neutrophils and eosinophils. The present study reinforces

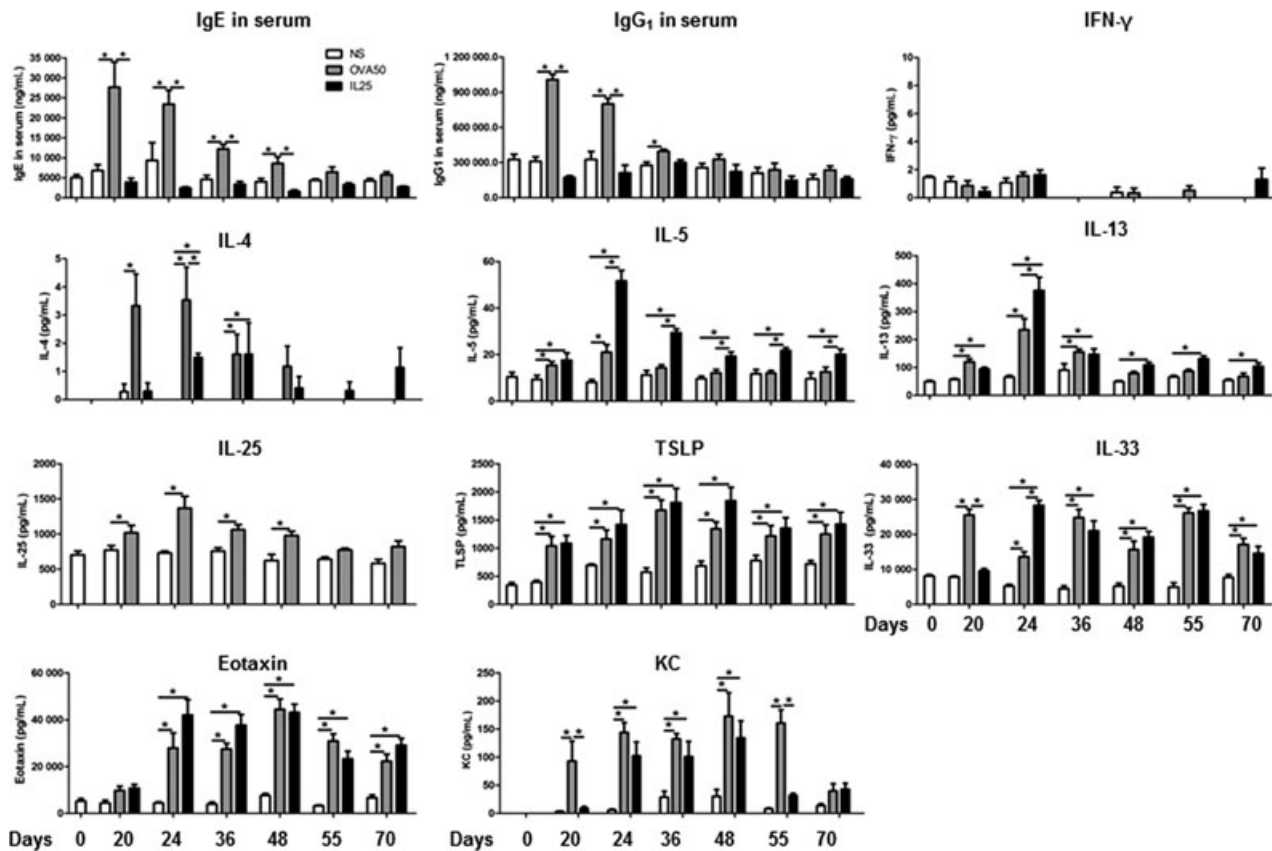


Fig. 7. Concentrations of cytokines and chemokines in lung homogenates from saline (NS), OVA- and IL-25-challenged mice at the time-points shown. Data were collected from three independent experiments and are expressed as mean $\pm$ SEM ($n = 5$ in each group at each time-point). * $P < 0.05$.

the impression that our observed effects of IL-25 are completely independent of IgE-mediated mechanisms. Although it has been shown that there is suppression of circulating, OVA-specific IgG₁ and IgE during OVA-induced airways inflammation in IL-25-deficient mice [26], IL-25, in stark contrast to OVA challenge in our experiments, did not elevate production of the IgE-switching cytokine IL-4 or total serum concentrations of IgG₁ or IgE, which is consistent with previous observations [27]. Finally, there is no evidence to suggest that IL-25 can cause direct degranulation of mast cells, although human mast cells in bronchial mucosal and skin biopsies have been reported to express its receptor [11]. Conversely, repeated OVA challenge clearly did result in local release of IL-25 into airways of the mice in these experiments, although how far this was a direct consequence of IgE-induced mast cell and/or basophil degranulation cannot be determined.

The peak of local eotaxin production induced by direct airways IL-25 challenge in our experiments coincided with the peak of eosinophil infiltration, suggesting that one important mechanism by which IL-25 causes eosinophil accumulation in the airways is

through secondary, local release of eotaxin and perhaps other, related chemokines. Previous studies [2, 16, 28] have also shown induction of local eotaxin production following IL-25 challenge of murine airways, while IL-25 is known to increase eotaxin expression by human fibroblasts, at least *in vitro* [14].

A number of cytokines have been implicated in contributing to goblet cell hyperplasia and mucus hypersecretion, both characteristic of human asthma. In our experiments, mucous hyperplasia was observed much earlier in the OVA-challenged compared with the IL-25-challenged animals, suggesting that other cytokines such as IL-4, not prominently induced by IL-25 alone, and IL-13 may be more relevant to the genesis of mucous hyperplasia in the context of allergen sensitization and challenge. IL-25-induced mucosal hypertrophy was attenuated in IL-13, IL-4R α , STAT-6 gene-deleted mice [16], while blockade of IL-25 in the course of IgE-mediated airways challenge reduced mucus secretion as well as IL-13 production [7]. Thus, while IL-25 may induce mucous hypertrophy through direct effects, it almost certainly also acts through secondary release of other cytokines, in particular IL-13.

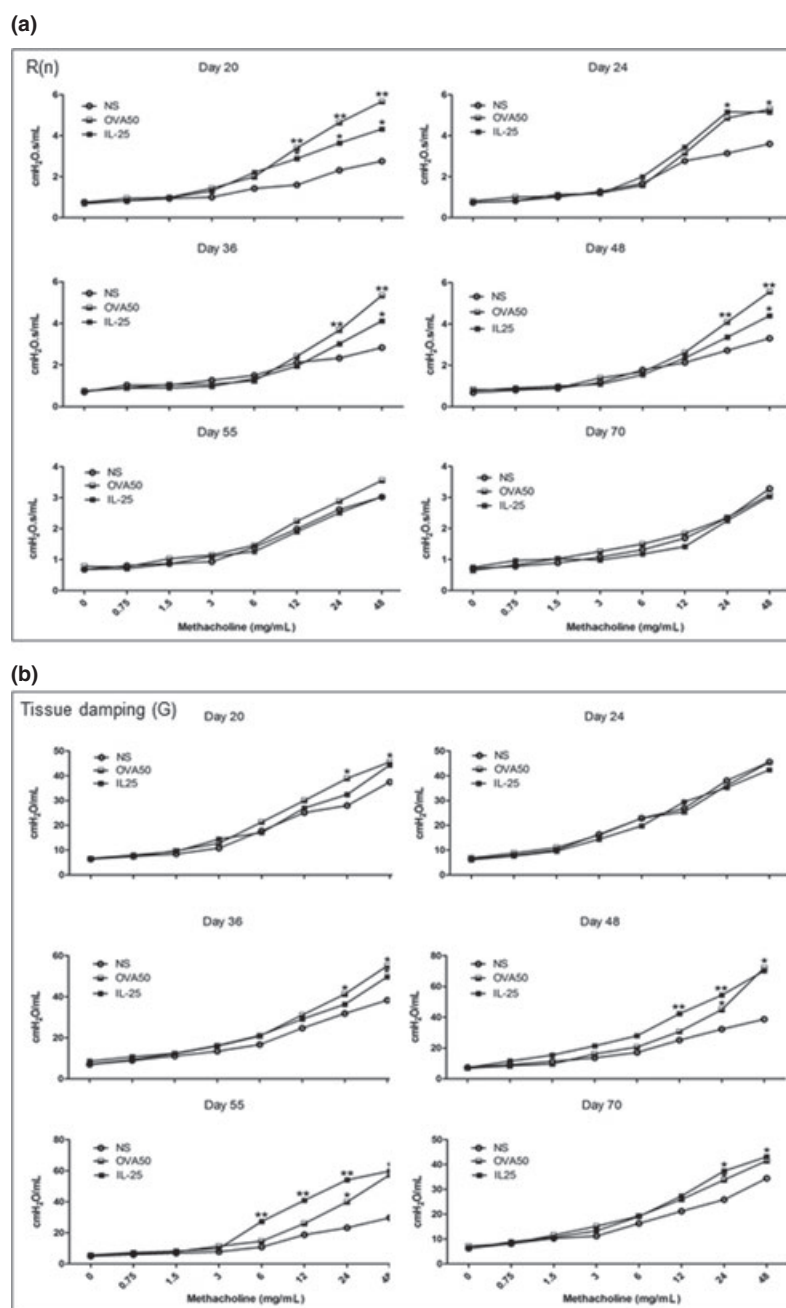


Fig. 8. (a) Central airways resistance (Rn) in saline (NS)-, OVA- and IL-25-challenged mice at the time-points. $**P < 0.05$ vs. IL-25- and NS-challenged mice. $*P < 0.05$ vs. NS-challenged mice. (b) Corresponding changes in tissue damping (G). Data were collected from three independent experiments and are expressed as the mean ($n = 5$ in each group at each time-point). $**P < 0.05$ vs. OVA-/or NS-challenged mice. $*P < 0.05$ vs. NS-challenged mice.

Deposition of collagen and other structural proteins into the interstitium of the airways mucosa is another recognized feature of airways remodelling in human asthma. Our study is the first directly to demonstrate that IL-25 increases collagen deposition in the lungs of animals, although in a previous study [8] it was demonstrated that, in mice repeatedly inhaling house dust mite protein with smad 2 in an adenoviral vector,

IL-25 blockade reduced collagen deposition. Again it cannot be determined from our data whether this is a direct or an indirect effect of IL-25. Certainly, IL-25 can act directly on human fibroblasts to induce collagen production [8, 14, 15], but on the other hand, increased local lay down of interstitial proteins may be effected by infiltrating eosinophils [29, 30]. In our experiments, IL-25-induced eosinophil infiltration of

the airways was observed to precede collagen lay down.

Interleukin-25 airways challenge alone also increased airways smooth muscle thickness, as has previously been observed in other murine, IgE-dependent repetitive challenge protocols such as with house dust mite [8] or OVA [31]. This may be partly a consequence of increased interstitial fibrosis, because previous studies have suggested that myofibroblasts might contribute to smooth muscle production [32]. Finally, as with repetitive airways house dust mite challenge of previously sensitized animals [8], direct application of IL-25 increased airways angiogenesis, which is consistent with our previous studies showing that IL-25 enhances proliferation of blood vascular endothelial cells and angiogenesis *in vitro* by up-regulating expression of bFGF, VEGF and their receptors [10, 12], although IL-25-induced angiogenesis in the present experiment could in theory result from the actions of IL-25 on a range of target cells. A number of cell types have been reported to express the IL-25 receptor, including inflammatory cells (T cells, NKT, ILCs, eosinophils, mast cells and basophils) and structural cells (such as endothelial cells, fibroblasts, smooth muscle cells and possibly epithelial cells) [33]. Therefore, IL-25, by acting on these cells, may directly or indirectly contribute to the pathogenesis of asthma, either in airways inflammation, remodelling or both. Additionally, other subtypes of T helper cells such as Th1, Th9 and Th17 cells and their products may also contribute to the pathogenesis of asthma [34].

Interleukin-33 and thymic structural lymphopoietin (TSLP) are two further innate, partially epithelially derived mediators which may also play an intermediary role in the effects of IL-25 on the airways. In our experiments, both IL-25 and OVA challenge increased expression of IL-33 and TSLP which persisted until the end of the experiments. This is consistent with previously observed reduction of IL-33 and TSLP in the airways of mice repeatedly challenged with house dust mite in the presence of anti-IL-25 antibody [8]. On the other hand, in a recent study [35] of repetitive 'allergen' challenge, IL-33 gene-deleted mice were less prone to develop airways hyperresponsiveness than IL-25 gene-deleted animals, suggesting that IL-33 plays a more significant complementary, rather than an intermediary role in this regard.

In this study, we chose to measure lung function using the invasive FlexiVent system; invasive systems

are thought to be more discriminatory of airways changes involving remodelling [36]. We observed that chronic exposure of murine airways to IL-25 increased hypersensitivity to methacholine inhalation, but also that, as time and the numbers of challenges progressed, the airways become progressively less susceptible to cholinergic bronchoconstriction. Correspondingly, as AHR declined, tissue damping (G) progressively increased. G is a measure of energy dissipated into lung tissues and is considered to be closely related to tissue resistance [37]. Our data suggest that it may be reflective of airways remodelling.

It is still not clear precisely what stimulates IL-25 production in asthmatic airways: environmental factors such as inhaled proteases [38] or viral infection [39] have been implicated in some studies. Our findings suggest here that increased IL-25 expression *per se* is sufficient to cause the airways inflammation, hyperresponsiveness and remodelling characteristic of asthma, and although IL-25 may act through multiple intermediary mediators, it may also act directly on specific target cells expressing its receptor. Either way, IL-25 would appear to be a key molecular target for asthma therapy, regardless of associated IgE-dependent mechanisms.

Acknowledgements

We thank the financial supports from the National Natural Science Foundation of China (81102250, 81170039, 81373177), the Science and Technology Project of the Beijing Municipal Education Commission (KM201110025005), PhD Programs Foundation of Ministry of Education of China (20091107110006, 20101107110003), Key Projects in the National Science & Technology Pillar Program during the Twelfth Five-year Plan Period (2012BAI05B02), AstraZeneca China Respiratory Research Awards 2011, the DANA Foundation, Asthma UK and the Friends of Guy's Hospital, London, UK. The authors also acknowledge financial support from the Department of Health via the National Institute for Health Research (NIHR) comprehensive Biomedical Research Centre award to Guy's & King's College London and King's College Hospital NHS Foundation Trust.

Conflict of interest

The authors declared no conflict of interest.

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