

Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma

R. K. Kumar, C. Herbert, M. Yang*, A. M. L. Koskinen*, A. N. J. McKenzie† and P. S. Foster*

Inflammation Research Unit, Department of Pathology, University of New South Wales, Sydney, Australia, *Department of Biochemistry and Molecular Biology, John Curtin School of Medical Research, Australian National University, Canberra, Australia, and †Medical Research Council Laboratory of Molecular Biology, Cambridge, UK

Summary

Background Interleukin-13 is believed to be important in asthmatic inflammation and airway hyper-reactivity.

Objective To investigate the role of IL-13 in chronic asthma, using an improved experimental model of asthma that reproduces most of the morphological features of the human disease.

Methods BALB/c mice or gene-targeted mice deficient in their ability to produce IL-13 or the IL-4 receptor α -chain (IL-4R α) were sensitized to ovalbumin and exposed to aerosolized antigen for 30 min/day on 3 days/week for 6 weeks. Intraepithelial eosinophils, accumulation of chronic inflammatory cells in the airway wall, subepithelial fibrosis, epithelial hypertrophy and numbers of mucous cells were quantified histomorphometrically. Airway hyper-reactivity (AHR) to a cholinergic agonist was assessed by barometric plethysmography.

Results Compared with wild-type animals, IL-13^{-/-} mice exhibited diminished accumulation of eosinophils and chronic inflammatory cells, as well as reduced subepithelial fibrosis, epithelial hypertrophy and mucous cell hyperplasia ($P < 0.01$ for all comparisons). In contrast, AHR was still demonstrable in IL-13^{-/-} mice. In IL-4R α ^{-/-} mice the inflammatory response, subepithelial fibrosis and AHR were similar to wild-type mice, although the receptor-deficient mice had significantly less epithelial hypertrophy and mucous cell hyperplasia.

Conclusion These results imply a critical role for IL-13 in accumulation of intraepithelial eosinophils in chronic asthma, as well as in epithelial and subepithelial remodelling. In addition, they suggest that in chronic asthma, IL-13 may be capable of signalling via a pathway that does not involve IL-4R α .

Keywords allergy, cytokines, eosinophils, *in vivo* animal models, inflammation

Submitted 1 October 2001; revised 3 January 2002; accepted 30 January 2002

Introduction

Asthma is a disorder characterized by episodic airflow obstruction and hyper-reactivity of the airways to a variety of stimuli [1]. Although most asthma is associated with atopy, the pathological basis of the clinical features of asthma remains poorly defined. However, it has been suggested that acute inflammation is primarily responsible for episodic bronchoconstriction, while chronic inflammation and airway wall remodelling contribute to airway hyper-reactivity (AHR) and to the fixed airflow obstruction that develops in chronic asthmatics [2].

With respect to acute allergic inflammation of the airways, animal studies in short-term high-level exposure models of bronchopulmonary inflammation suggest an important role for Th2 cytokines such as interleukins 4, 5 and 13, which are primarily produced by CD4⁺ T-lymphocytes [3–11]. However, much less is known about the pathogenesis of chronic

inflammation and airway wall remodelling in asthma. A major reason for this has been the lack of suitable animal experimental models of chronic human asthma that replicate these features of the disease [12, 13].

We have described an improved model of chronic asthmatic inflammation of the airways in mice [14]. This involves systemic sensitization of high IgE-responder BALB/c mice to ovalbumin and subsequent chronic inhalational challenge with low levels of aerosolized antigen for 30 min/day, 3 days/week. In contrast to other reports in which long-term challenge is associated with down-regulation of airway inflammation and hyper-reactivity [15, 16], this approach consistently elicits lesions typical of chronic human asthma. These include: accumulation of numerous intraepithelial eosinophils; chronic inflammation in the lamina propria with accumulation of lymphocytes, plasmacytoid lymphocytes and macrophages; and epithelial hypertrophy, mucous cell hyperplasia/metaplasia and subepithelial fibrosis. The inflammatory changes are maximal after 6 weeks of inhalational exposure. Importantly, and in sharp contrast to virtually all previously reported murine experimental models of asthma, there is no evidence of alveolitis associated with the

Correspondence: R. K. Kumar, Department of Pathology, University of New South Wales, Sydney, Australia 2052. E-mail: R.Kumar@unsw.edu.au

airway lesions. In addition, the mice exhibit marked airway hyper-reactivity to an inhaled cholinergic agonist, which can be attributed to abnormalities of the airways rather than to parenchymal lesions. Thus this model replicates most of the features that are typical of the chronic human disease [17].

Ongoing studies in models of acute allergic bronchopulmonary inflammation have provided considerable evidence in support of an important role for IL-13 in asthma. Expression of IL-13 is up-regulated after local antigen challenge of asthmatics [18]. IL-13 has been shown to directly induce eosinophil recruitment and airway hyper-reactivity in mice, while inhibition of the biological activity of IL-13 has been demonstrated to be associated with diminution of eosinophil recruitment, airway hyper-reactivity and mucus production [8, 9]. Using mice genetically deficient in IL-13, we have shown that IL-13 acts in concert with IL-4 and IL-5 to modulate inflammation and hyper-reactivity [19]. Furthermore, studies in transgenic mice with high pulmonary expression of IL-13 demonstrated induction of airway hyper-reactivity and various changes of remodelling [20].

Accumulating evidence from studies in humans indicates that there are potentially multiple cellular sources of IL-13, including CD4⁺ T lymphocytes, basophils and mast cells [21]. Signalling mechanisms for IL-13 are beginning to be unravelled. Current evidence suggests that the IL-4 receptor α -chain (IL-4R α) plays an important role in most IL-13 receptor complexes and may therefore be a key target for therapeutic intervention in asthma [22].

To test the hypothesis that IL-13 plays a crucial role in inflammation, airway wall remodelling and airway hyper-reactivity in chronic asthma, we examined these responses in mice genetically deficient in IL-13 or IL-4R α using our improved experimental model. Our results establish that IL-13 is important both in accumulation of eosinophils and in airway wall remodelling in chronic disease, but indicate that its absence does not prevent development of airway hyper-reactivity. Furthermore, our findings strongly suggest that in chronic asthma, IL-13 may be capable of signalling via a pathway that does not involve IL-4R α .

Methods

Animals

Wild-type specific pathogen-free female BALB/c mice were obtained from the Biological Resources Centre, University of New South Wales. Mice genetically deficient in their ability to produce IL-13 (IL-13^{-/-}) were generated from (129 $\times$ C57BL/6) mice [23] that were backcrossed for six generations on to the BALB/c strain [19]. These mice, together with BALB/c mice deficient in their ability to express the IL-4R α chain (IL-4R α ^{-/-}) [24], were obtained from the Animal Services Division, Australian National University. Animals were aged approximately 8 weeks at the commencement of experimental studies. They were maintained in a laminar flow holding unit (Gelman Sciences, Sydney) and housed in autoclaved cages on autoclaved bedding in an air conditioned room on a 12-h light/dark cycle. Irradiated food and acidified water were provided *ad libitum* throughout. All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of the University of New South Wales (98/29).

Sensitization and inhalational exposure

This was performed essentially as previously described [14], except that, to ensure reproducible induction of an antiovalbumin IgE response, mice were sensitized by an intraperitoneal injection of 50 μ g of alum-precipitated chicken egg ovalbumin (Grade V, $\geq$ 98% pure, Sigma, St Louis, MO, USA; unless otherwise specified, all chemicals were obtained from this source) 21 days and 7 days (booster injection) before inhalational exposure. Experimental groups comprising six to eight immunized and boosted animals were exposed to aerosolized ovalbumin for 30 min/day on 3 days/week for 6 weeks in a whole-body inhalation exposure system (Unifab Corporation, Kalamazoo, MI, USA). During the exposure, the animals were held in wire flow-through cage racks and filtered air was drawn through the 0.5 m³ inhalation chamber at a flow rate of 250 L/min.

A solution of 2.5% ovalbumin in normal saline was aerosolized by delivery of compressed air to a sidestream jet nebuliser (Trimed, Sydney, Australia) and injected into the airstream entering the chamber. The concentration and size distribution of particles within the breathing zone of the mice were continuously and cumulatively monitored using a light scatter device (PCAM-TX, PPM, Knoxville, TN, USA). Particle concentration was maintained in the range 10–20 mg/m³ by controlling the airflow into the nebuliser. Control groups were non-immunized non-exposed (i.e. naïve) wild type and gene-targeted animals. Because we have previously demonstrated [25] that exposure of non-immunized animals to aerosolized antigen for 6 weeks is not associated with development of significant airway inflammation, remodelling or AHR, the use of naïve animals instead of non-immunized exposed animals as controls was appropriate.

Collection of tissues

Tissues for histopathological examination were collected 18 h after the last inhalational exposure and fixed in 10% buffered formalin overnight. The longitudinally orientated trachea and a horizontal slice from the mid-zone of the single-lobed left lung were embedded in paraffin. Ribbons of two to three sections cut at 5 μ m were stained with haematoxylin & eosin for assessment of inflammatory changes in sections of the longitudinally orientated trachea, with Gordon & Sweet's reticulin stain for assessment of collagen deposition, or with Alcian blue-PAS for enumeration of mucin secreting cells in the intrapulmonary conducting airways.

Morphometry

The validity and reliability of the morphometric techniques we employed for quantifying inflammation and remodelling have been established in previous reports [14, 25]. To enumerate inflammatory cells, images of haematoxylin & eosin-stained sections were captured with a Spot Cooled Color Digital camera (Diagnostic Instruments, Sterling Heights, MI, USA), which was calibrated with a reference measurement slide. Nuclear profiles in the lamina propria were counted in every second consecutive microscopic field and the length of the epithelial basement membrane was measured by tracing using the Spot software. The data were used to calculate the mean number of cells per 100 μ m of epithelial basement membrane in the lamina propria for individual animals.

In parallel, the full length of the epithelial surface was examined using a $\times 100$ oil immersion objective to enumerate intraepithelial eosinophils, which were readily identified on the basis of their strongly staining cytoplasmic granules. The data were expressed as number of cells per mm of epithelial basement membrane for individual animals. The thickness of the epithelial layer was also measured in these sections and the mean thickness for each animal was calculated.

Reticulin-stained sections were used for assessment of subepithelial collagenization. The thickness of the reticulin-stained zone underneath the tracheal epithelium was measured and the mean thickness for each animal was calculated.

Metaplasia and/or hyperplasia of mucus-secreting goblet cells was assessed in sections of lung tissue stained with Alcian blue-PAS. Goblet cells are rarely present in the epithelium of the intrapulmonary airways of normal BALB/c mice [26] and their presence was thus readily apparent in these sections. The percentage of cells staining positive for mucins was enumerated in the largest visible airway and each animal was categorized according to a semilogarithmic scale as grade 0 = < 1% positive cells, grade 1 = 1 to 3%, grade 2 = 4 to 10%, grade 3 = 11 to 30% and grade 4 = $\geq 31\%$.

Airway reactivity

Responsiveness to methacholine was assessed in conscious, unrestrained mice by barometric plethysmography, using apparatus and software supplied by Buxco (Troy, NY, USA). This system yields a dimensionless parameter known as enhanced Pause (Penh), reflecting changes in waveform of the pressure signal from the plethysmography chamber combined with a timing comparison of early and late expiration, which can be used to empirically monitor airway function. Measurement was performed essentially as previously described [27]. Briefly, mice were placed in the chamber and baseline readings taken and averaged for 3 min. Aerosolized methacholine (concentrations in solution ranging from 3.125 to 50 mg/mL) was then delivered through an inlet into the chamber for 3 min and readings averaged over a period of 3 min after each dose was administered.

Statistical analysis

Results of morphometry and assessment of airway reactivity are presented as arithmetic mean $\pm$ standard error for each experimental group. In general, an initial one-way analysis of variance followed by Newman-Keuls multiple comparison test was used to examine differences between groups. Comparison of the proportion of mucin-positive cells in the intrapulmonary airways was based upon grading and therefore employed a non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison procedure. The software package GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used for all data analysis and preparation of graphs.

Results

Airway inflammation

As previously described [14], sensitized wild-type BALB/c mice exhibited accumulation of significant numbers of eosinophils within the epithelial layer following repeated exposure to

aerosolized antigen. Similarly, accumulation of intraepithelial eosinophils was readily apparent in IL-4R α ^{-/-} mice (Fig. 1a). The relative number of eosinophils within the epithelium was comparable in these two groups and was significantly increased compared with the corresponding naïve animals

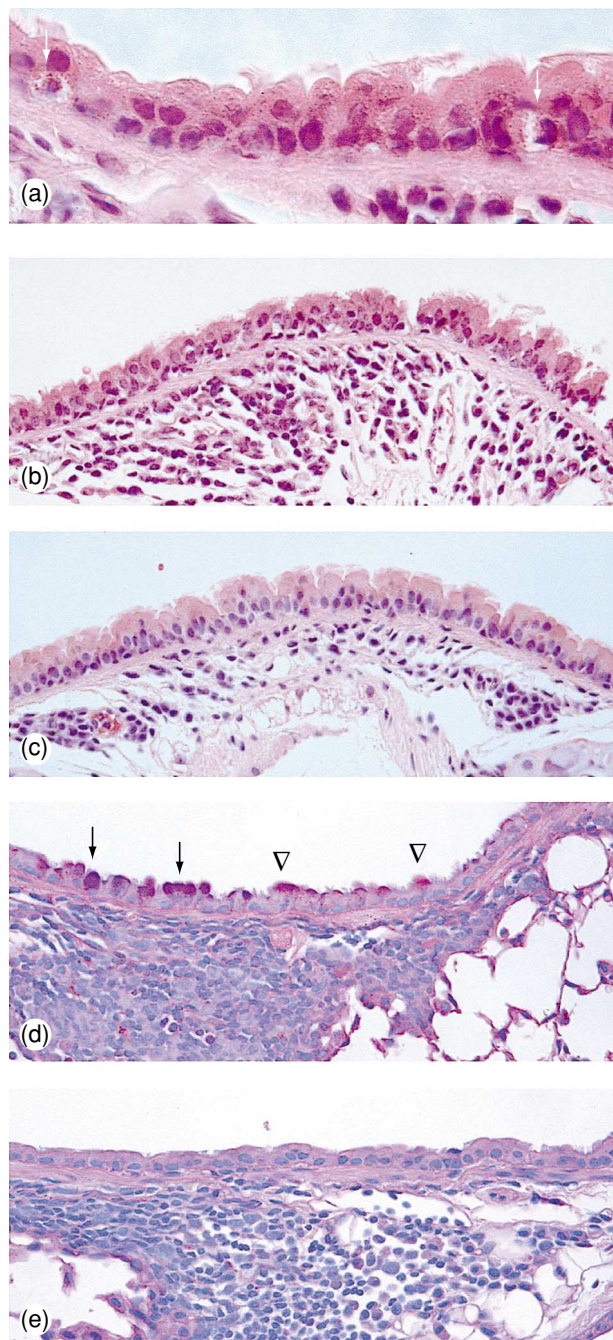


Fig. 1. (a) Eosinophils within the tracheal epithelium of a sensitized, chronically exposed IL-4R α ^{-/-} animal (arrows) stained with haematoxylin & eosin. (b) Prominent aggregates of chronic inflammatory cells in the lamina propria of the trachea of a sensitized, chronically exposed wild-type BALB/c mouse. (c) Reduced accumulation of chronic inflammatory cells in an IL-13-deficient mouse. (d) Numerous mucous cells in the intrapulmonary airways of a sensitized, chronically exposed wild-type BALB/c mouse, stained with Alcian Blue (arrows) or PAS (arrowheads). Note the chronic inflammatory response in the airway wall. (e) Absence of goblet cell hyperplasia/metaplasia in an IL-13-deficient animal.

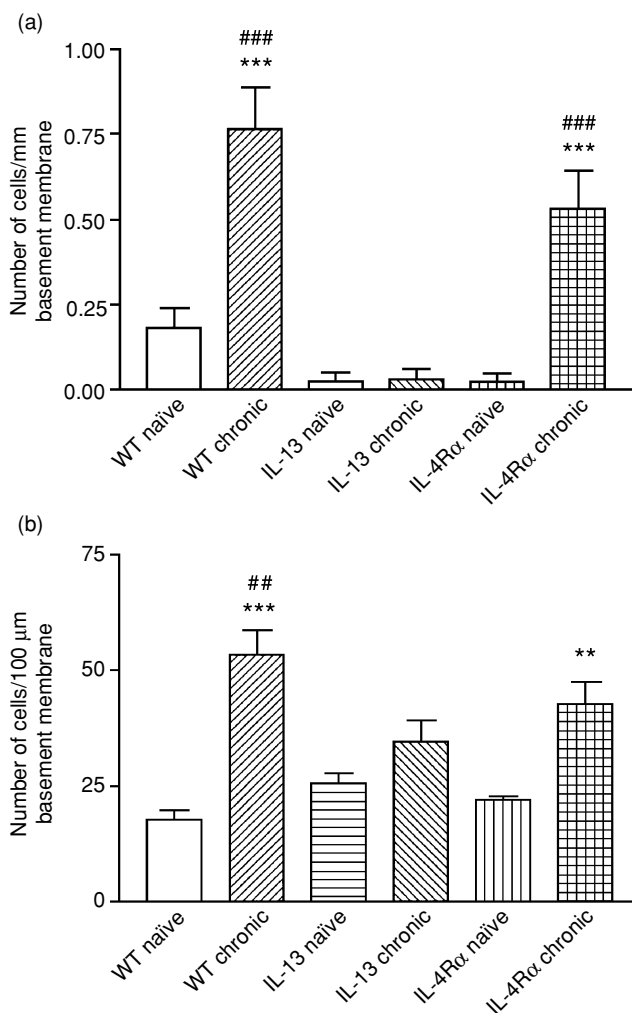


Fig. 2. (a) Intraepithelial eosinophils and (b) inflammation in the lamina propria of the trachea of naïve and sensitized, chronically exposed wild-type, IL-13^{-/-} and IL-4Rα^{-/-} mice. Significant differences compared with corresponding naïve animals are shown as ** $P < 0.01$, *** $P < 0.001$; significant differences compared with chronically exposed IL-13^{-/-} animals are shown as ## = $P < 0.01$, ### = $P < 0.001$.

($P < 0.001$) (Fig. 2a). In contrast, accumulation of eosinophils was virtually abolished in IL-13-deficient mice. The reduction in intraepithelial eosinophils in IL-13-deficient mice relative to both wild-type and IL-4Rα^{-/-} mice was significant ($P < 0.001$ for both comparisons).

In sensitized chronically exposed wild-type mice, there was a statistically significant increase in the mean profile density of cells in the airway wall as a consequence of accumulation of lymphocytes, plasmacytoid lymphocytes and other mononuclear inflammatory cells (Fig. 1b) ($P < 0.001$ compared with naïve wild-type mice, Fig. 2b). A lesser, but still statistically significant, accumulation of chronic inflammatory cells was also evident in the airways of IL-4Rα^{-/-} mice ($P < 0.01$ compared with naïve controls, Fig. 2b). Fewer chronic inflammatory cells were recruited into the lamina propria in IL-13^{-/-} mice (Fig. 1c). The reduction in chronic inflammatory cell accumulation in IL-13-deficient mice was significant compared with wild-type mice ($P < 0.01$) but not compared with IL-4Rα^{-/-} mice (Fig. 2b).

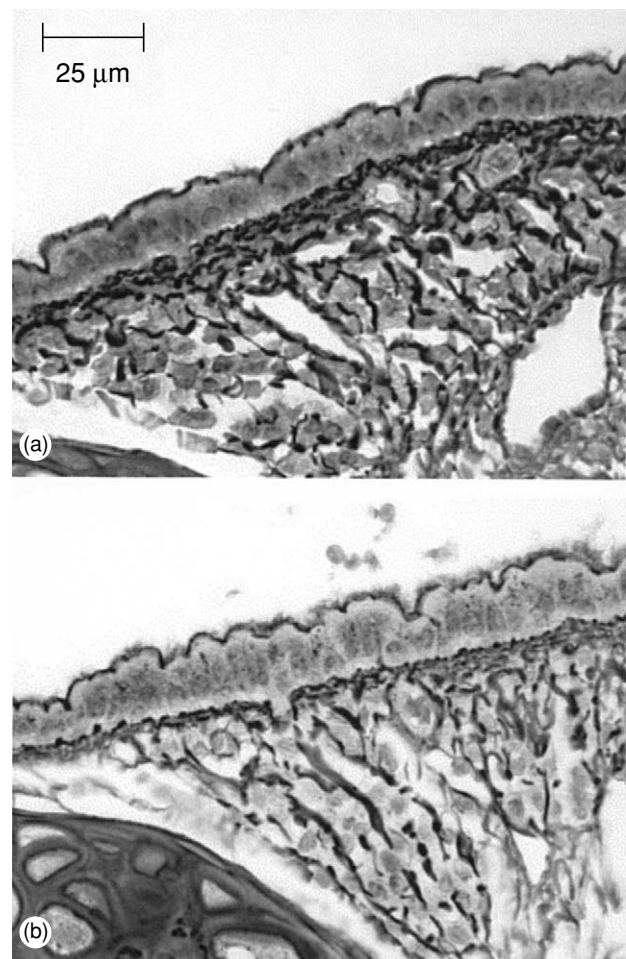


Fig. 3. (a) A prominent zone of reticulin-stained subepithelial collagen was apparent in tracheas of sensitized, chronically exposed wild-type mice. (b) The reticulin-stained zone in IL-13-deficient mice was much thinner and was equivalent to that observed in naïve mice.

Subepithelial fibrosis

As shown in Fig. 3(a), a prominent subepithelial reticulin-stained zone is apparent in sensitized wild-type mice exposed to antigen. Compared with control animals, the mean thickness of this zone was significantly increased ($P < 0.001$) (Fig. 4a). In IL-13-deficient mice, subepithelial collagenization did not occur and the thickness of the reticulin-stained zone was equivalent to that in naïve wild-type and gene-targeted animals (Fig. 3b). In contrast, collagenization in IL-4Rα^{-/-} mice was comparable with wild-type mice and significantly increased compared with the corresponding naïve receptor-deficient mice ($P < 0.001$) (Fig. 4a). Relative to both wild-type and IL-4Rα^{-/-} mice, the reduction in subepithelial fibrosis in IL-13-deficient mice was significant ($P < 0.001$ for both comparisons).

Epithelial changes

The mean thickness of the airway epithelium was significantly increased in sensitized wild-type BALB/c mice after 6 weeks' inhalation exposure to antigen ($P < 0.01$ compared with naïve control animals) (Fig. 4b). In contrast, there was no evidence of hypertrophy of epithelial cells in either IL-13^{-/-} or IL-4Rα^{-/-} mice following chronic inhalational exposure to aerosolized

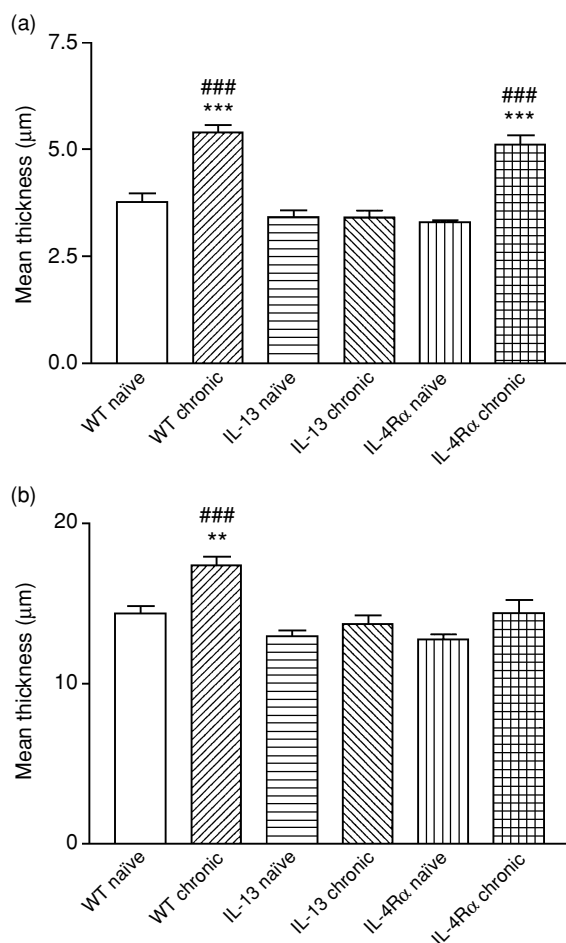


Fig. 4. (a) Subepithelial collagenization and (b) hypertrophy of the tracheal epithelium in naïve and sensitized, chronically exposed wild-type, IL-13^{-/-} and IL-4Rα^{-/-} mice. Significant differences compared with corresponding naïve animals are shown as ** $P < 0.01$, *** $P < 0.001$; significant differences compared with chronically exposed IL-13^{-/-} animals are shown as ### $P < 0.001$.

ovalbumin. Epithelial thickness was significantly less than in wild-type mice in both of these groups ($P < 0.001$) (Fig. 4b).

Mucus-secreting goblet cells were virtually absent in the intrapulmonary airways of naïve wild-type mice and this was also true of naïve IL-13^{-/-} and IL-4Rα^{-/-} animals, so that on morphometric quantification the mean grade recorded for these groups was zero. Consistent with our previous study [14], there was a striking increase in the number of goblet cells in the intrapulmonary airways of sensitized exposed wild-type BALB/c mice (Fig. 1d), with a mean grade of 3.56 ± 0.24 . No such increase was demonstrable in either IL-13^{-/-} (Fig. 1e) or IL-4Rα^{-/-} mice. The mean grades for these latter two groups were, respectively, 0.88 ± 0.35 and 1.00 ± 0.27 , which were significantly lower than wild-type mice ($P < 0.001$ in the case of IL-13^{-/-} mice and $P < 0.01$ in the case of IL-4Rα^{-/-} mice).

Airway responsiveness

As reported previously, exposure to increasing concentrations of aerosolized methacholine increased Penh values in naïve wild-type mice [25]. The increase was approximately fivefold relative to baseline at a concentration of 50 mg/mL (Fig. 5a). Sensitized exposed wild-type animals exhibited a left-shifted dose-response curve and increased maximal reactivity, characteristic of airway hyper-reactivity (Fig. 5a). Compared with controls, the increase in Penh was significantly greater at methacholine concentrations of 12.5, 25 and 50 mg/mL. Naïve IL-13^{-/-} mice exhibited much greater responsiveness to methacholine than naïve wild-type animals, with a greater than 10-fold increase in Penh relative to baseline at 50 mg/mL (significantly greater than wild type mice, $P < 0.01$). Nevertheless, sensitized exposed IL-13^{-/-} mice had an even more markedly left-shifted dose-response curve and were still demonstrably hyper-reactive to methacholine, with significantly greater Penh than naïve animals at concentrations of 12.5 and 25 mg/mL (Fig. 5b). In contrast, naïve IL-4Rα^{-/-} animals were similar to naïve wild-type mice in their response to methacholine, with a 4.5-fold increase in Penh at 50 mg/mL compared

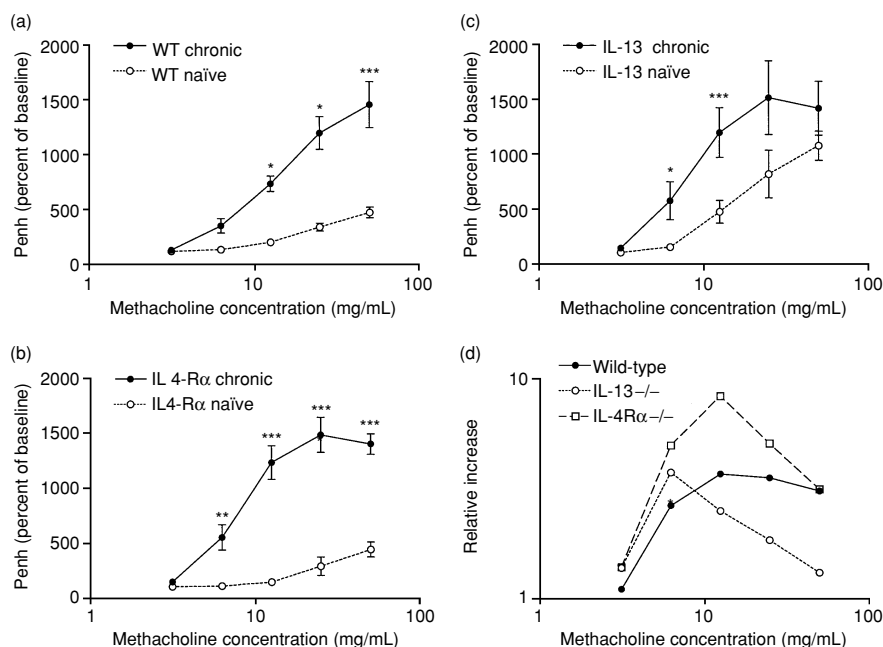


Fig. 5. Airway reactivity of naïve and sensitized chronically exposed mice, assessed by change in Penh in response to increasing concentrations of aerosolized β -methacholine. (a) Wild-type mice. (b) IL-13^{-/-} mice. (c) IL-4Rα^{-/-} mice. Significant differences compared with naïve animals are shown as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (d) Relative increase in Penh compared with naïve controls for all three strains at each concentration of methacholine.

with baseline, while sensitized exposed IL-4R α $-/-$ mice had a dose-response curve very similar to that of IL-13 $-/-$ mice and their increase in Penh was significantly greater than naïve controls at all methacholine concentrations above 3.125 mg/mL (Fig. 5c). The relative increase in Penh compared with naïve controls for all three strains at each concentration of methacholine is shown in Fig. 5(d).

Discussion

In this study, we investigated the role of IL-13 in the pathogenesis of chronic asthma using an improved murine model of allergic inflammation of the airways. This model allowed us not only to examine the contribution of IL-13 to allergic inflammation and airway hyper-reactivity, but also the induction of chronic inflammation and remodelling of the airways. Because these animals do not develop a significant alveolitis, relatively few inflammatory cells are present in bronchoalveolar lavage fluid and lavage is thus an inappropriate method of quantifying inflammation in this model. We therefore employed morphometric quantification of eosinophils within the airway epithelium and of chronic inflammatory cells within the lamina propria.

Development of the characteristic morphological lesions of chronic asthma was strikingly down-regulated in animals deficient in their ability to produce IL-13 as a result of gene targeting. Specifically, IL-13-deficient mice exhibited markedly reduced intraepithelial accumulation of eosinophils, moderately decreased numbers of chronic inflammatory cells in the airway wall, absence of both subepithelial collagenization and epithelial thickening, as well as markedly decreased mucous cell hyperplasia/metaplasia. However, these animals still demonstrated residual airway hyper-reactivity to a cholinergic agonist.

There is evidence that IL-13 induces airway epithelial expression of eotaxin, which is a major chemoattractant for eosinophils [11]. In addition, IL-13-mediated eosinophil recruitment appears to be substantially dependent on IL-5 [28]. Although it might be expected that eosinophil accumulation would be diminished in IL-13 $-/-$ mice, our previous studies using a short-term high exposure model of allergic bronchopulmonary inflammation did not yield evidence in support of this prediction [19]. A possible explanation is that the florid inflammatory response in the lung parenchyma in the acute exposure model activates IL-13-independent pathways for eosinophil recruitment, such as those responsible for the marked eosinophilia that is observed in peripheral blood in acutely exposed animals [29]. In contrast, the airway-specific inflammatory changes in the chronic exposure model employed in this study are clearly regulated by IL-13 in a critically important manner. We speculate that IL-13 may similarly be important in the regulation of eosinophil recruitment into the airways in chronic human asthma.

The numerical density of chronic inflammatory cells in the lamina propria was significantly decreased in sensitized chronically exposed IL-13 $-/-$ mice as compared with wild-type mice. The mechanisms responsible for the decreased number of chronic inflammatory cells are currently undefined. However, immunohistochemistry revealed that immunoglobulin-synthesizing cells, which comprise a substantial proportion of

the inflammatory cells in the lamina propria [30], were undiminished (data not shown).

Our data provide unambiguous evidence of a role for IL-13 in airway wall remodelling in chronic asthma. Of particular interest is the finding that subepithelial fibrosis was virtually absent in IL-13 $-/-$ mice. This distinctive lesion of human asthma is well reproduced in our chronic inhalation exposure model [14] but is not amenable to study in animal models of acute allergic bronchopulmonary inflammation. Our observations are concordant with data from a model of granulomatous inflammation and fibrosis following *Schistosoma mansoni* infection, in which IL-13 was shown to be a profibrotic cytokine [31, 32]. They are also consistent with the demonstration that pulmonary overexpression of the IL-13 gene using a lung-specific promoter is associated with peribronchiolar fibrosis [20]. A possible mechanism for this action of IL-13 is its ability to directly stimulate proliferation of fibroblasts [33, 34].

Epithelial remodelling was also strikingly down-regulated in IL-13 $-/-$ mice. The virtual absence of goblet cell metaplasia in the intrapulmonary airways of these animals was not unexpected, as intratracheal administration of IL-13 rapidly induces this change in the airways of wild-type mice and similar down-regulation of this short-term response has been observed in acute exposure models using inhibitors of IL-13 [8, 9]. An additional finding to emerge from the use of the chronic inhalation exposure model was that epithelial hypertrophy was also diminished in IL-13 $-/-$ mice. Recent studies have emphasized the potential role of ligands for the epidermal growth factor receptor in airway wall remodelling in human asthma [35, 36], particularly with respect to epithelial injury and repair. However, immunohistochemical studies did not yield any evidence of diminished expression of epidermal growth factor, TGF- α or amphiregulin by airway epithelial cells in IL-13 $-/-$ mice (unpublished data), suggesting that altered expression of these growth factors was unlikely to account for the epithelial changes.

Despite the lack of intraepithelial accumulation of eosinophils and the absence of remodelling, IL-13 $-/-$ mice continued to exhibit modest hyper-reactivity to inhaled β -methacholine relative to naïve controls. Interestingly, the reactivity of IL-13 $-/-$ control animals to methacholine was significantly augmented. Both of these findings are consistent with our previously reported results in a short-term high exposure model of allergic bronchopulmonary inflammation [19], in which naïve IL-13-sufficient control mice, similarly backcrossed from (129 $\times$ C57BL/6) on to the BALB/c strain, also exhibited enhanced reactivity to methacholine. However, they contrast with the observations of Wills-Karp et al. [8], who have suggested that IL-13 is the primary CD4 $^{+}$ T cell-derived factor responsible for AHR.

A possible explanation for this discrepancy is the induction in IL-13 $-/-$ mice of other as yet undefined cytokine pathways that could stimulate AHR, e.g. IL-13 is a potent regulator of macrophage function and production of IL-12 may be increased in IL-13 $-/-$ mice [23]. Our findings are also markedly at variance with the recently reported results of Walter et al. [37], who used IL-13 $-/-$ mice of the same origin, backcrossed on to the BALB/c strain for one more generation, and reported no change in the responsiveness of naïve animals to methacholine, together with absence of AHR in sensitized acutely challenged animals. The difference may be attributable to different sensitization and challenge regimes, which in our hands may lead to a more

robust IL-4 response. At least in the short-term model, this persistent IL-4 response in IL-13^{-/-} animals appears to contribute to AHR, because reactivity to methacholine is strikingly decreased if sensitized exposed IL-13^{-/-} mice are pre-treated with anti-IL-4 [19].

There was a remarkable discordance between the inflammatory changes observed in IL-13^{-/-} and IL-4R α ^{-/-} mice in this model of chronic asthma. The IL-4R α ^{-/-} mice were not significantly different from wild-type mice with respect to accumulation of intraepithelial eosinophils or of chronic inflammatory cells in the lamina propria. Because absence of IL-13 appears to be rate-limiting for development of eosinophilic inflammation, it is possible that in the IL-4R α knockout mice, IL-13 signalling is occurring via a receptor or receptors that do not include the IL-4R α chain. This inference is supported by our recent studies showing that transfer of antigen-specific T cells from IL-13-sufficient mice induced AHR and eosinophilic inflammation in IL-4R α -deficient recipient animals, whereas T cells from IL-13-deficient mice did not [38]. Validation of this concept in a model of chronic asthma has important implications for the proposed therapeutic targeting of the IL-4R α chain in human asthma.

Although IL-4R α ^{-/-} mice developed subepithelial collagenization comparable with wild-type mice, they did not exhibit a similar degree of epithelial thickening. In our previously reported studies of wild-type and cytokine gene-deficient mice, epithelial hypertrophy and subepithelial fibrosis have consistently developed in parallel [14, 25]. The lack of epithelial hypertrophy in IL-4R α ^{-/-} mice implies a role for this receptor chain in a signalling mechanism controlling the epithelial response. Furthermore, it is clear that in this model of chronic asthma, as in other models [39], the IL-4R α chain plays an important role in cytokine signalling that regulates mucous cell metaplasia and hyperplasia.

We previously reported that while airway inflammation in IL-4^{-/-} mice was comparable with wild-type mice, the IL-4-deficient animals exhibited increased epithelial thickening and subepithelial collagenization following chronic inhalation exposure to allergen [25]. The results of the present study, which demonstrate marked diminution of both airway inflammation and remodelling in IL-13^{-/-} mice, emphasize the different functions of these two Th2 cytokines in chronic asthma and may have important implications for the development of novel therapeutic strategies appropriate to human asthma.

Acknowledgements

This work was supported by grants from the National Health and Medical Research Council of Australia (to R. K. K.) and the Human Frontiers Science Program (to P. S. F.). We thank Angelina Enno and Gavin McKenzie for invaluable assistance in the preparation and staining of tissue sections.

References

- Chung KF, Adcock I. Asthma: application of cell and molecular biology techniques to unravel causes and pathophysiological mechanisms. In: Chung KF, Adcock I, eds. *Asthma: Mechanisms and Protocols*. Totowa, New Jersey: Humana Press Inc., 2000; 1–29.
- Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola A. Asthma: from bronchoconstriction to airways inflammation and remodeling. *Am J Respir Crit Care Med* 2000; 161:1720–45.
- Garlisi CG, Falcone A, Kung TT et al. T cells are necessary for Th2 cytokine production and eosinophil accumulation in airways of antigen-challenged allergic mice. *Clin Immunol Immunopathol* 1995; 75:75–83.
- Eum SY, Haile S, Lefort J, Huerre M, Vargaftig BB. Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. *Proc Natl Acad Sci USA* 1995; 92:12290–4.
- Foster PS, Hogan SP, Ramsay AJ, Matthei KI, Young IG. Interleukin 5 deficiency abolishes eosinophilia, airways hyperreactivity, and lung damage in a mouse asthma model. *J Exp Med* 1996; 183:195–201.
- Corry DB, Folkesson HG, Warnock ML et al. Interleukin 4, but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperreactivity. *J Exp Med* 1996; 183:109–17.
- Kaminuma O, Mori A, Ogawa K et al. Successful transfer of late phase T cell eosinophil infiltration in the lung by infusion of helper T cell clones. *Am J Respir Cell Mol Biol* 1997; 16:448–54.
- Wills-Karp M, Luyimbazi J, Xu X et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998; 282:2258–61.
- Grunig G, Warnock M, Wakil AE et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998; 282:2261–3.
- Hogan SP, Koskinen A, Matthei KI, Young IG, Foster PS. Interleukin-5-producing CD4⁺ T cells play a pivotal role in aeroallergen-induced eosinophilia, bronchial hyperreactivity, and lung damage in mice. *Am J Respir Crit Care Med* 1998; 157:210–8.
- Li L, Xia Y, Nguyen A et al. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J Immunol* 1999; 162:2477–87.
- Richards IM. Mouse models of allergic disease; how do they relate to asthma in man? *Clin Exp Allergy* 1996; 26:618–20.
- Nakamura H, Weiss ST, Israel E, Luster AD, Drazen JM, Lilly CM. Eotaxin and impaired lung function in asthma. *Am J Respir Crit Care Med* 1999; 160:1952–6.
- Temelkovski J, Hogan SP, Shepherd DP, Foster PS, Kumar RK. An improved murine model of asthma: selective airway inflammation, epithelial lesions and increased methacholine responsiveness following chronic exposure to aerosolised allergen. *Thorax* 1998; 53:849–56.
- Yiamouyiannis CA, Schramm CM, Puddington L et al. Shifts in lung lymphocyte profiles correlate with the sequential development of acute allergic and chronic tolerant stages in a murine asthma model. *Am J Pathol* 1999; 154:1911–21.
- Palmans E, Kips JC, Pauwels RA. Prolonged allergen exposure induces structural airway changes in sensitized rats. *Am J Respir Crit Care Med* 2000; 161:627–35.
- Kumar RK, Foster PS. Murine model of chronic human asthma. *Immunol Cell Biol* 2001; 79:141–4.
- Huang SK, Xiao HQ, Kleine-Tebbe J et al. IL-13 expression at the sites of allergen challenge in patients with asthma. *J Immunol* 1995; 155:2688–94.
- Webb DC, McKenzie ANJ, Koskinen AML, Yang M, Mattes J, Foster PS. Integrated signals between IL-13, IL-4, and IL-5 regulate airways hyperreactivity. *J Immunol* 2000; 165:108–13.
- Zhu Z, Homer RJ, Wang Z et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999; 103:779–88.
- Brombacher F. The role of interleukin-13 in infectious diseases and allergy. *Bioessays* 2000; 22:646–56.

- 22 Jensen PL. The interleukin 13 receptor complex. *Stem Cells* 2000; 18:61–2.
- 23 McKenzie GJ, Emson CL, Bell SE et al. Impaired development of Th2 cells in IL-13-deficient mice. *Immunity* 1998; 9:423–32.
- 24 Noben-Trauth N, Shultz LD, Brombacher F, Urban JF, Gu H, Paul WE. An interleukin (IL-4)-independent pathway for CD4+ T cell IL-4 production is revealed in IL-4 receptor-deficient mice. *Proc Natl Acad Sci USA* 1997; 94:10838–43.
- 25 Foster PS, Ming Y, Matthei KI, Young IG, Temelkovski J, Kumar RK. Dissociation of inflammatory and epithelial responses in a murine model of chronic asthma. *Lab Invest* 2000; 80:655–62.
- 26 Blyth DI, Pedrick MS, Savage TJ, Bright H, Beesley JE, Sanjar S. Induction, duration and resolution of airway goblet cell hyperplasia in a murine model of atopic asthma: effect of concurrent infection with respiratory syncytial virus and response to dexamethasone. *Am J Respir Cell Mol Biol* 1998; 19:38–54.
- 27 Hamelmann E, Schwarze J, Takeda K et al. Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am J Respir Crit Care Med* 1997; 156:766–75.
- 28 Pope SM, Brandt EB, Mishra A et al. IL-13 induces eosinophil recruitment into the lung by an IL-5- and eotaxin-dependent mechanism. *J Allergy Clin Immunol* 2001; 108:594–601.
- 29 Mould AW, Matthei KI, Young IG, Foster PS. Relationship between interleukin-5 and eotaxin in regulating blood and tissue eosinophilia in mice. *J Clin Invest* 1997; 99:1064–71.
- 30 Kumar RK, Temelkovski J, McNeil HP, Hunter N. Airway inflammation in a murine model of chronic asthma: evidence for a local humoral immune response. *Clin Exp Allergy* 2000; 30:1486–92.
- 31 Chiaramonte MG, Donaldson DD, Cheever AW, Wynn TA. An IL-13 inhibitor blocks the development of hepatic fibrosis during a T-helper type 2-dominated inflammatory response. *J Clin Invest* 1999; 104:777–85.
- 32 Fallon PG, Richardson EJ, McKenzie GJ, McKenzie AN. Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 and IL-13: IL-13 is a profibrotic agent. *J Immunol* 2000; 164:2585–91.
- 33 Doucet C, Brouty-Boye D, Pottin-Clemenceau C et al. Interleukin (IL) 4 and IL-13 act on human lung fibroblasts: implication in asthma. *J Clin Invest* 1998; 101:2129–39.
- 34 Kraft M, Lewis C, Pham D, Chu HW. IL-4, IL-13, and dexamethasone augment fibroblast proliferation in asthma. *J Allergy Clin Immunol* 2001; 107:602–6.
- 35 Davies DE, Polosa R, Puddicombe SM, Richter A, Holgate ST. The epidermal growth factor receptor and its ligand family: their potential role in repair and remodelling in asthma. *Allergy* 1999; 54:771–83.
- 36 Puddicombe SM, Polosa R, Richter A et al. Involvement of the epidermal growth factor receptor in epithelial repair in asthma. *FASEB J* 2000; 14:1362–74.
- 37 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.
- 38 Mattes J, Yang M, Siqueira A et al. IL-13 induces airways hyperreactivity independently of the IL-4R α chain in the allergic lung. *J Immunol* 2001; 167:1683–92.
- 39 Cohn L, Homer RJ, MacLeod H, Mohrs M, Brombacher F, Bottomly K. Th2-induced airway mucus production is dependent on IL-4R α , but not on eosinophils. *J Immunol* 1999; 162: 6178–83.