

Interleukin-17 Signalling in a Murine Model of Mild Chronic Asthma

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Key Words

Airway epithelial cells · Airway inflammation · Airway wall remodelling · Animal models · Asthma · Chronic allergen challenge · Th17 cells

Abstract

Background: The role of Th17 cell-derived cytokines in the pathogenesis of airway inflammation and remodelling in mild asthma remains unclear. We investigated this in a mouse model which reproduces most of the features of the human disease. **Methods:** Systemically sensitised BALB/c mice were challenged via the airways with a low mass concentration of ovalbumin aerosol for 8 weeks to induce lesions of mild chronic asthma. Changes were compared with those in animals deficient in signalling via the interleukin (IL)-17 receptor A (IL-17R). Low-passage airway epithelial cells (AEC) and fibroblasts were cultured with IL-17A, or with media from Th17-polarised cells, to assess activation. **Results:** In CD4⁺ T cells from chronically challenged mice, expression of mRNA for Th17 cytokines IL-17A, IL-17F, IL-21 and IL-22 was significantly increased. Both recombinant IL-17A and media from Th17 cells significantly stimulated the production of various pro-inflammatory and pro-remodelling cytokines by AEC and fibroblasts. In the mouse model, abrogation of IL-17R signalling had no effect on the development

of airway inflammation or on most changes of remodelling. However, numbers of mucus-producing cells and expression of mRNA for Gob-5 were attenuated in the absence of IL-17R signalling. **Conclusions:** Although IL-17A and Th17 cells stimulate cytokine production by structural cells of the airways, and Th17 cells are induced in our model of mild chronic asthma, signalling via IL-17R did not contribute significantly to the development of airway inflammation and most changes of remodelling in this model. However, in mild asthma, IL-17A appears to have a role in the goblet cell response in the airways.

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Introduction

Asthma is a chronic inflammatory disease of the airways associated with a variety of structural changes in the airway wall and hyper-responsiveness of the airways to bronchoconstrictor stimuli [1]. Although T helper type 2 (Th2) lymphocytes and their cytokines play a key role in the pathogenesis of lesions in a substantial proportion of patients [2], there is mounting evidence that other T helper subsets, such as Th17 cells, are also involved in this condition [3].

Differentiation of Th17 cells from naive precursors [4] is induced by interleukin (IL)-6 and transforming growth factor (TGF)- β and is dependent on the transcription factor ROR- γ t [5, 6]. Subsequent expansion of this population of cells is driven by IL-23 [7]. Th17 cells are characterised by the production of IL-17A [8] as well as IL-17F, IL-21, and IL-22 [9].

A number of studies have demonstrated that IL-17A has the potential to contribute to airway inflammation, in particular recruitment of neutrophils in severe asthma [10]. Elevated levels of IL-17A have been demonstrated in sputum, lavage fluid and plasma of asthmatics [11, 12], and IL-17A-positive cells have also been identified in the airway wall [13].

A few studies have also suggested that IL-17A may promote the development of airway wall remodelling. Both IL-17A and IL-17F bind to the IL-17 receptor A (IL-17R) and can stimulate the production of cytokines and growth factors by airway epithelial cells (AEC) [14]. Th17 cytokines have multiple effects on airway smooth muscle cells, including inducing migration and enhancing contractile force [15, 16]. Wang et al. [17] reported that Th17 cells promoted the expression of heparin-binding epidermal growth factor (HB-EGF), which contributed to both proliferation of airway smooth muscle and mucus production in a chronic challenge model of asthma in mice. A recent study in gene-targeted mice unable to generate Th17 cells is also consistent with a role for these cells in airway remodelling [18].

However, *in vivo* studies to date have employed models of severe ongoing allergic inflammation. The precise role of Th17 cells and IL-17A in the development of the inflammation and remodelling of the airway wall associated with mild to moderate chronic asthma therefore remains unclear. To investigate this, we utilised our well-characterised model of chronic asthma in BALB/c mice, which are systemically sensitised to ovalbumin (OVA) and repeatedly challenged with a low mass concentration (≈ 3 mg/m³) of aerosolised OVA for 8 weeks. Chronic challenge induces acute-on-chronic inflammation of the airways, as well as changes of remodelling such as sub-epithelial fibrosis and goblet cell metaplasia [19]. Importantly, low-level challenge induces minimal parenchymal inflammation so that, unlike in other models, lesions are confined to the conducting airways and our model thus closely resembles mild chronic human asthma [20]. In this model, we have previously reported increased expression of mRNA for IL-17A in the airways, consistent with expansion of a population of cells with a Th17 phenotype [21]. In the current study, we investigated the expression

of IL-17A and other Th17-related cytokines by pulmonary CD4⁺ T cells in our model. In parallel, we assessed *in vitro* production of pro-inflammatory and pro-remodelling cytokines by low-passage cultures of primary mouse AEC and fibroblasts in response to IL-17A and Th17 cells. Furthermore, we defined the role of signalling via IL-17R in this model of chronic asthma using IL-17R-deficient mice.

Materials and Methods

Mice, Sensitisation and Challenge

Specific pathogen-free wild-type (WT) BALB/c animals were obtained from the Biological Resources Centre of the University of New South Wales (UNSW). IL-17R-deficient animals on a BALB/c background were kindly provided by Amgen (Thousand Oaks, Calif., USA). Hemizygous offspring were crossed and homozygous WT and IL-17R-deficient breeding pairs were established. Mice were housed in individually ventilated cages with autoclaved bedding on a 12-hour light/dark cycle. Autoclaved food and water were provided *ad libitum* throughout. Animals aged 7–8 weeks were sensitised and challenged with chicken egg OVA (grade V, $\geq 98\%$ pure; Sigma, Australia; unless otherwise specified, all chemicals were from this source) as previously described [19]. Briefly, mice received 2 intra-peritoneal injections of 50 μ g of alum-precipitated OVA at 21 and 7 days prior to the inhalational challenge; they were then exposed to a controlled low mass concentration of ≈ 3 mg/m³ of aerosolised OVA for 30 min/day, 3 days/week, for 8 weeks (fig. 1) in a whole-body inhalation exposure system (Unifab Corporation, Kalamazoo, Mich., USA). Particle concentration was continuously monitored using a light scatter device (Dust Track 8520; TSI, St. Paul, Minn., USA). All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of the UNSW (ref. 08/114B).

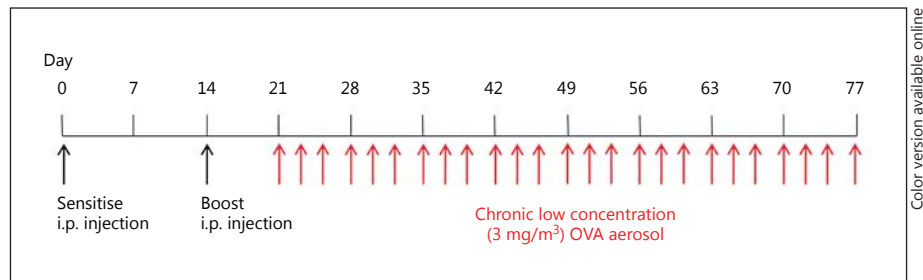
Cell and Tissue Collection

Animals were sacrificed 24 h after the final inhalational challenge. Proximal airway tissue was collected by blunt dissection as previously described [21] and samples were homogenised in TRI reagent for extraction of mRNA. For isolation of CD4⁺ T cells, lung tissue was disaggregated with collagenase and magnetic bead separation was performed as previously described [22].

Histopathological Assessment

For histopathology, the trachea was cannulated and the left lung was inflated with 10% neutral buffered formalin. Paraffin-embedded sections were stained with haematoxylin and eosin for enumeration of inflammatory cells, with Gordon and Sweet's reticulin stain for assessment of collagen deposition, or with Alcian Blue/PAS for assessment of mucous cells. Morphometric assessment of airway inflammation and remodelling was performed using our well-validated methods, as previously described [19, 23]. Inflammation in the conducting airways was assessed by enumeration of intra-epithelial eosinophils and of nuclear profiles in the lamina propria of the trachea. Neutrophils in lung tissue were identified by immunohistochemical staining using a rat anti-Gr-1 monoclonal antibody (RB6-8C5; Pharmingen, San Diego, Calif., USA). The thickness of the sub-epithelial zone of collagenisation

Fig. 1. Timeline of murine model of chronic asthma. Animals received 2 intra-peritoneal (i.p.) injections of 50 µg of alum-precipitated OVA at 21 and 7 days prior to the inhalational challenge and were then exposed to a controlled low mass concentration of $\approx 3 \text{ mg/m}^3$ of aerosolised OVA for 30 min/day, 3 days/week, for 8 weeks. Animals were sacrificed 24 h after the final inhalational challenge.



was measured in the trachea, together with the thickness of the airway epithelium. Metaplasia and/or hyperplasia of mucus-secreting goblet cells was assessed in lung sections using a semi-logarithmic grading scale.

Culture of AEC and Fibroblasts

AEC and fibroblasts were established from explants of trachea and lung tissue from BALB/c mice as previously described [24]. Primary mouse lung fibroblasts were grown in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, Calif., USA) supplemented with 10% foetal calf serum. Primary AEC were grown in serum-free MCDB201 with 0.5% fatty acid-free bovine serum albumin, 30 mg/ml of transferrin (Roche Applied Sciences, Castle Hill, N.S.W., Australia), 30 µg/ml of EGF, 20 ng/ml of insulin-like growth factor (IGF)-1 (GroPep Bioreagents, Adelaide, S.A., Australia), and other supplements as described. Cultures were maintained in an atmosphere of 5% CO₂ in air. To assess responses to Th17 cytokines, cells were cultured in serum-free medium without growth factors for 24 h and then stimulated with 5 or 50 ng/ml of recombinant IL-17A (R&D Systems, Minneapolis, Minn., USA) or with 25% conditioned media for a further 24 h.

Culture of Th1, Th2 and Th17 Cells

CD4⁺ T cells were purified from spleens of BALB/c mice and expanded in RPMI-1640 media containing 10% foetal calf serum in the presence of appropriate cytokines, antibodies and CD3/CD28 activation beads to generate cells of the Th1, Th2 or Th17 phenotype. A Th1 phenotype was induced using IL-2 (10 ng/ml), IL-12 (10 ng/ml) and anti-IL-4 (10 µg/ml); a Th2 phenotype was generated with IL-2 (10 ng/ml), IL-4 (10 ng/ml) and anti-interferon (IFN)-γ (10 µg/ml), and a Th17 phenotype was induced with IL-1β, IL-6, IL-23 (20 ng/ml of each), TGF-β₁ (1 ng/ml) and both anti-IL-4 and anti-IFN-γ (10 µg/ml of each). A population of CD4⁺ T cells nonspecifically stimulated with CD3/CD28 beads was used as a negative control. After 4 days of differentiation in culture, cells were washed and resuspended in fresh medium, and supernatants were collected 24 h later. The phenotype of T cells was confirmed by assessing mRNA and protein expression of IFN-γ, IL-5 and IL-17A using quantitative real-time PCR and a multiplex immunoassay.

Assessment of Cytokine Expression

Extracted mRNA was treated with DNase (TurboDNase; Ambion, Austin, Tex., USA) and reverse transcribed using Superscript III (Invitrogen). SYBR green-based quantitative real-time PCR was used to assess expression of inflammatory cytokines and growth factors. Primers were custom-designed in house to allow

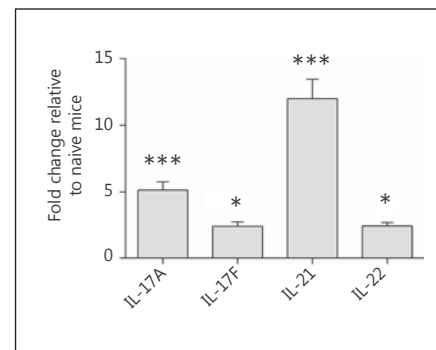


Fig. 2. Expression of Th17 cytokines in a model of chronic asthma. Relative expression of mRNA for various Th17 cytokines by purified CD4⁺ T-lymphocytes from chronically challenged WT BALB/c animals. Significant differences compared to cells from naive mice are shown as * $p < 0.05$ and *** $p < 0.001$.

the use of identical thermocycler conditions, permitting simultaneous assessment of multiple cytokines. Reactions were performed on a Roche Light Cycler 480 (Roche Diagnostics Corporation, Indianapolis, Ind., USA) and expression was normalised to HPRT. The pro-inflammatory cytokines assessed were IL-1β, IL-6, IL-11, tumour necrosis factor (TNF)-α, granulocyte-macrophage colony-stimulating factor (GM-CSF) and the chemokine CXCL1. The growth factors assessed were IGF-1, platelet-derived growth factor, fibroblast growth factor (FGF)-1, FGF-2, EGF, TGF-α, amphiregulin, HB-EGF, TGF-β₁, connective tissue growth factor and vascular endothelial growth factor (VEGF).

Cytokine Assays

The concentration of cytokines in cell culture supernatant was measured using a Bio-Plex assay (Mouse 23-plex panel: Bio-Rad Laboratories, Hercules, Calif., USA) according to the manufacturer's instructions.

Statistical Analysis

Results are presented as means ± standard error. Differences between groups were assessed using a one-way ANOVA or Kruskal-Wallis test, followed by a Tukey, Dunnett or Dunn post-test as appropriate. The software package GraphPad Prism (version 5.04; GraphPad Software, San Diego, Calif., USA) was used for all analyses and preparation of graphs.

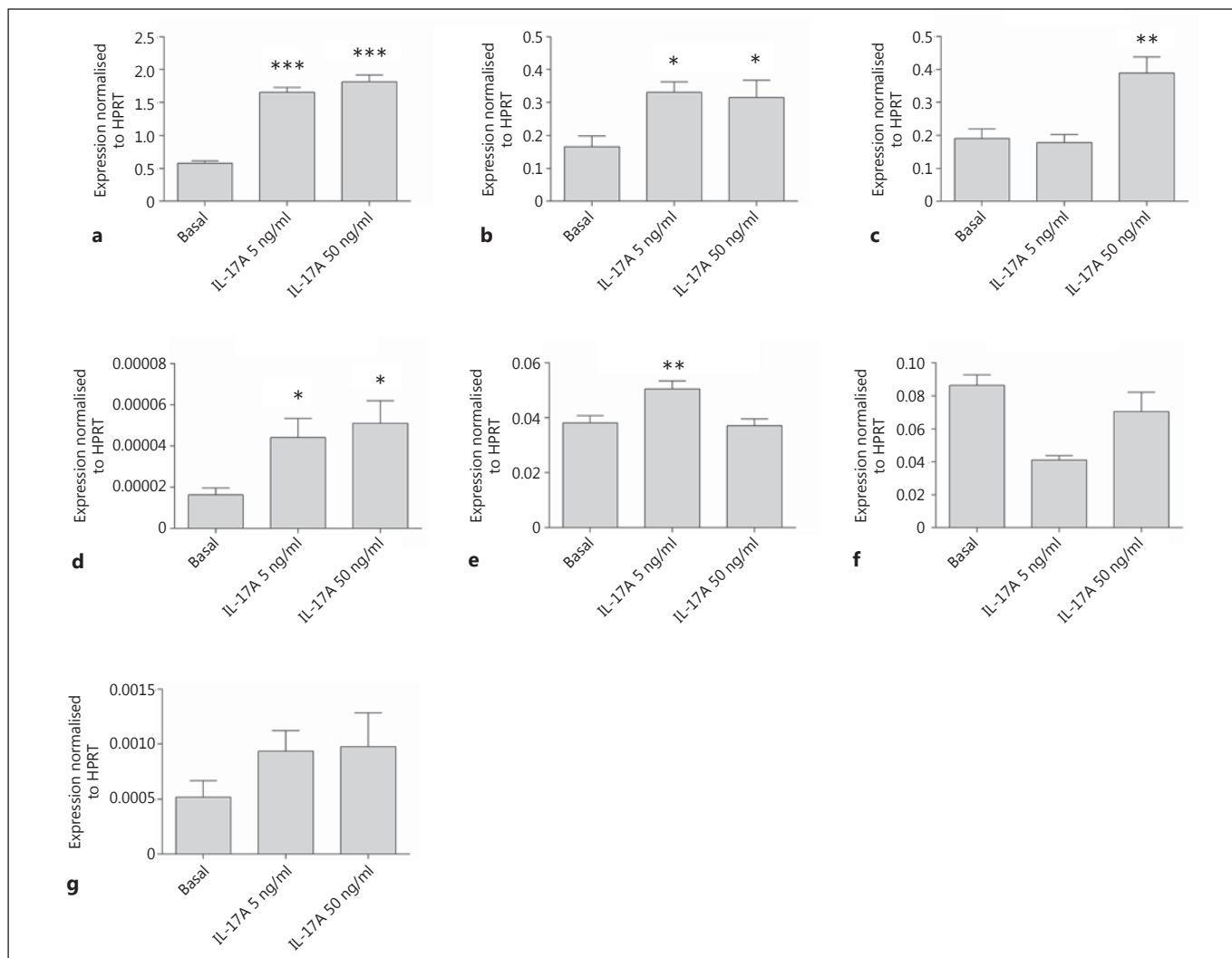


Fig. 3. Expression of mRNA by AEC cultured with IL-17A (5 and 50 ng/ml) normalised to HPRT. **a** CXCL-1. **b** IL-6. **c** IGF-1. **d** GM-CSF. **e** HB-EGF. **f** VEGF. **g** Gob-5. Data are means $\pm$ SEM. Significant differences compared to cells cultured with basal media are shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Results

In vivo Evidence of Th17 Cells in Chronic Asthmatic Inflammation

To determine whether Th17 cells participated in the development of inflammation in this model of chronic asthma, we assessed cytokine expression by purified pulmonary CD4⁺ T cells without re-stimulation. CD4⁺ T lymphocytes from sensitised, chronically challenged mice exhibited significant increases in the expression of mRNA for key Th17 cell cytokines, including IL-17A, IL-17F, IL-21 and IL-22 (fig. 2).

In vitro Evidence of the Role of Th17 Cytokines in Inflammation and Remodelling

To assess the potential role of IL-17A and related cytokines, we undertook two sets of experiments.

Effects of Stimulation with Recombinant IL-17A

We stimulated primary AEC and fibroblasts, cultured from tissue explants, with recombinant IL-17A (5 and 50 ng/ml). In AEC, there was a significant and marked increase in the expression of mRNA for the inflammatory cytokines CXCL1 and GM-CSF, as well as an increase in the expression of IL-6 (fig. 3). This was paralleled by in-

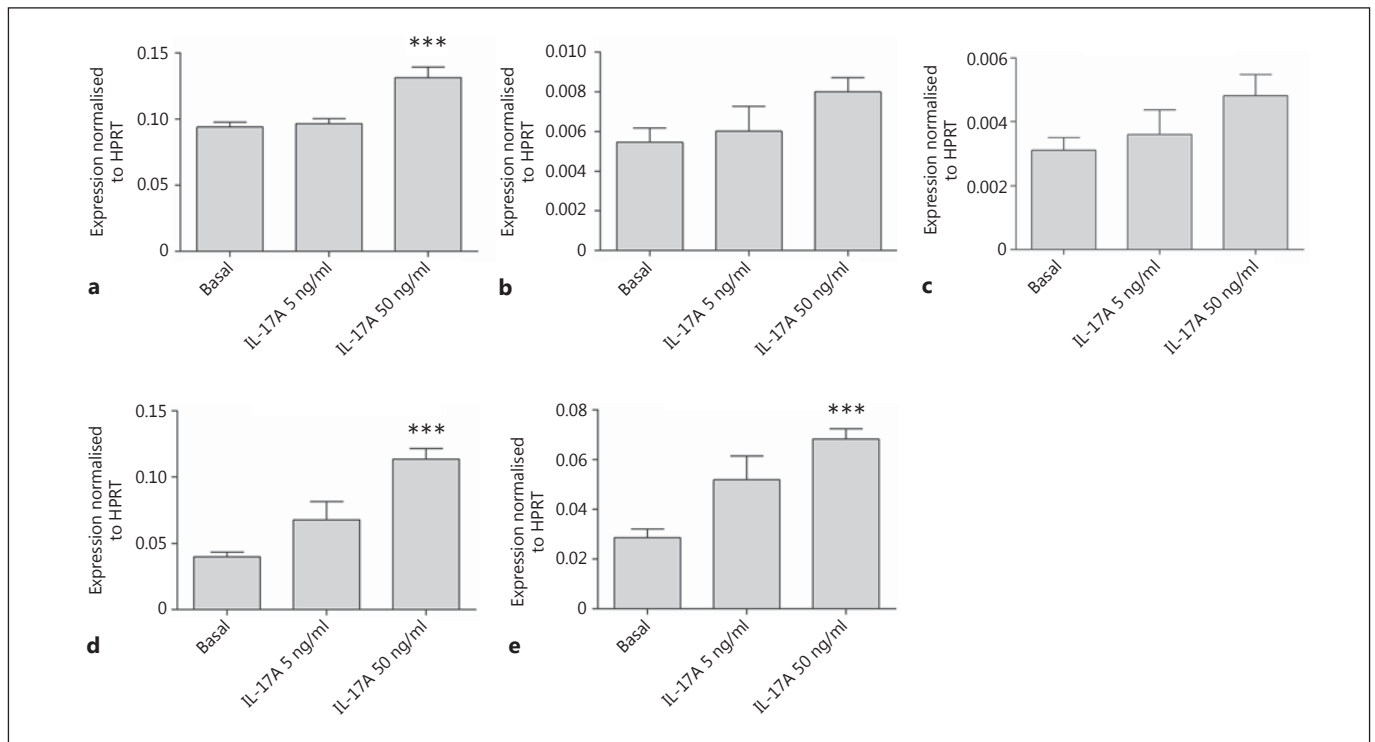


Fig. 4. Expression of mRNA for remodelling related cytokines and growth factors by fibroblasts cultured with IL-17A (5 and 50 ng/ml) normalised to HPRT. **a** IL-6. **b** IL-11. **c** FGF-1. **d** FGF-2. **e** HB-EGF. Data are means $\pm$ SEM. Significant differences compared to cells cultured with basal media are shown as *** $p < 0.001$.

creased expression of mRNA for the remodelling-related growth factor IGF-1 (fig. 3). Other growth factors examined, such as HB-EGF and VEGF, did not exhibit consistently increased expression. In addition, there was no increase in mRNA for the chloride channel Gob-5, associated with mucous cell differentiation, although its expression was in any case very low in these undifferentiated immersion cultures of AEC.

The effects of IL-17A on mouse lung fibroblasts were different from those on AEC. There was a significant increase in the expression of mRNA for IL-6, FGF-2 and HB-EGF (fig. 4).

Effects of Stimulation with Th17-Conditioned Medium

To further investigate whether cytokines produced by Th17 cells could drive the production of pro-inflammatory and pro-fibrotic cytokines by structural cells of the airways, T cells polarised towards a Th17 phenotype were generated in vitro, together with cells polarised towards a Th1 or Th2 phenotype for comparison (table 1). Condi-

tioned media from naive or polarised T cells were added to AEC or fibroblasts in culture, and the production of pro-inflammatory and pro-fibrotic cytokines was assessed.

Relative to the effects of media from control T cells, AEC treated with media from Th17 cells exhibited significantly increased expression of mRNA for CXCL1, IL-6 and GM-CSF, as well as HB-EGF and VEGF (table 2). In addition, there was an increase in mRNA for Gob-5, although this was not statistically significant. We found, however, that these effects were not specific to Th17-conditioned media, as AEC were stimulated to a comparable extent by media from cells polarised to a Th2 phenotype.

In contrast, fibroblasts treated with media from Th17 cells exhibited significant increases in the expression of IL-6, IL-11 and FGF-2, as well as increased expression of FGF-1 which was not statistically significant, whereas no such increases were observed when fibroblasts were treated with media from Th2 cells (table 3).

Table 1. Expression of mRNA and protein by in vitro polarised Th1, Th2 and Th17 cells

	IFN- γ		IL-5		IL-17A	
	mRNA	protein	mRNA	protein	mRNA	protein
Th1	3.02 $\pm$ 0.27***	6.14 $\pm$ 0.29***	0.18 $\pm$ 0.03	0.28 $\pm$ 0.03	0.60 $\pm$ 0.18	0.44 $\pm$ 0.07
Th2	0.67 $\pm$ 0.05	0.45 $\pm$ 0.07	1.83 $\pm$ 0.23*	1.53 $\pm$ 0.12**	1.39 $\pm$ 0.35	0.89 $\pm$ 0.09
Th17	0.96 $\pm$ 0.12	0.64 $\pm$ 0.08	1.32 $\pm$ 0.34	0.78 $\pm$ 0.07	123.27 $\pm$ 27.49***	18.1 $\pm$ 4.26***

Fold change in expression of mRNA and protein production for cytokines in polarised Th1, Th2 and Th17 cell subsets.

Data are means $\pm$ SEM. Significant differences compared to naive T cells are shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 2. Expression of mRNA by AEC cultured with conditioned media

Cytokine mRNA	Th1	Th2	Th17
CXCL1	2.91 $\pm$ 0.20	2.12 $\pm$ 0.10	14.63 $\pm$ 1.56***
IL-6	2.38 $\pm$ 0.19	6.60 $\pm$ 0.45***	8.47 $\pm$ 0.80***
IGF-1	0.37 $\pm$ 0.03**	1.10 $\pm$ 0.09	0.79 $\pm$ 0.06
GM-CSF	3.07 $\pm$ 0.78*	4.27 $\pm$ 0.65**	5.56 $\pm$ 0.50***
HB-EGF	1.30 $\pm$ 0.05*	1.69 $\pm$ 0.04***	1.40 $\pm$ 0.02**
VEGF	1.70 $\pm$ 0.08**	1.99 $\pm$ 0.18***	2.27 $\pm$ 0.17***
Gob-5	0.68 $\pm$ 0.20	1.47 $\pm$ 0.88	2.80 $\pm$ 0.71

Fold change in expression of mRNA for cytokines and growth factors by AEC treated with conditioned media from Th1, Th2 and Th17 cells. Data are means $\pm$ SEM. Significant differences compared to cells incubated with media from naive T cells are shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

Table 3. Expression of mRNA by fibroblasts cultured with conditioned media

Cytokine mRNA	Th1	Th2	Th17
IL-6	1.24 $\pm$ 0.12	1.00 $\pm$ 0.06	1.48 $\pm$ 0.07**
IL-11	0.67 $\pm$ 0.05	0.85 $\pm$ 0.11	1.77 $\pm$ 0.36*
FGF-1	1.02 $\pm$ 0.16	0.91 $\pm$ 0.10	2.30 $\pm$ 0.77
FGF-2	0.85 $\pm$ 0.05	0.94 $\pm$ 0.10	1.31 $\pm$ 0.07*

Fold change in expression of mRNA for remodelling related cytokines and growth factors by fibroblasts cultured with conditioned media from Th1, Th2 and Th17 cells. Data are means $\pm$ SEM. Significant differences compared to cells incubated with media from naive T cells are shown as * $p < 0.05$ and ** $p < 0.01$.

None of the other cytokines or growth factors examined was significantly upregulated in either cell type.

In vivo Evidence of the Role of Th17 Cytokines in Inflammation and Remodelling

To assess the importance of signalling via the IL-17A receptor in the development of pathological changes in the airways, we compared responses of IL-17R-deficient mice in a model of chronic asthma.

Effects on Airway Inflammation

We found no evidence that IL-17R deficiency had any effect on the accumulation of intra-epithelial eosinophils or of chronic inflammatory cells in the lamina propria of the airways of chronically challenged mice. Marked increases in cell numbers were observed in both groups, but there was no significant difference between WT and IL-17R-deficient animals (fig. 5a, b). Similarly, the num-

bers of Gr-1-positive neutrophils in lung tissue were comparably increased in both groups (fig. 5c). Furthermore, in both WT and IL-17R-deficient animals, expression of mRNA for a variety of pro-inflammatory cytokines, including CXCL1, TNF- α , IFN- γ , IL-4 and IL-13, was elevated following chronic allergen challenge and again there were no significant differences between these groups (fig. 6).

Effects on Airway Remodelling

In contrast to the findings with respect to airway inflammation, IL-17R deficiency did have an effect on the development of changes in airway remodelling in this low-level chronic challenge model. Whereas epithelial thickening and sub-epithelial fibrosis were both comparable in WT and IL-17R-deficient animals (fig. 7a, b), the significant increase in the number of mucus-positive cells in the airways observed in WT animals following chronic

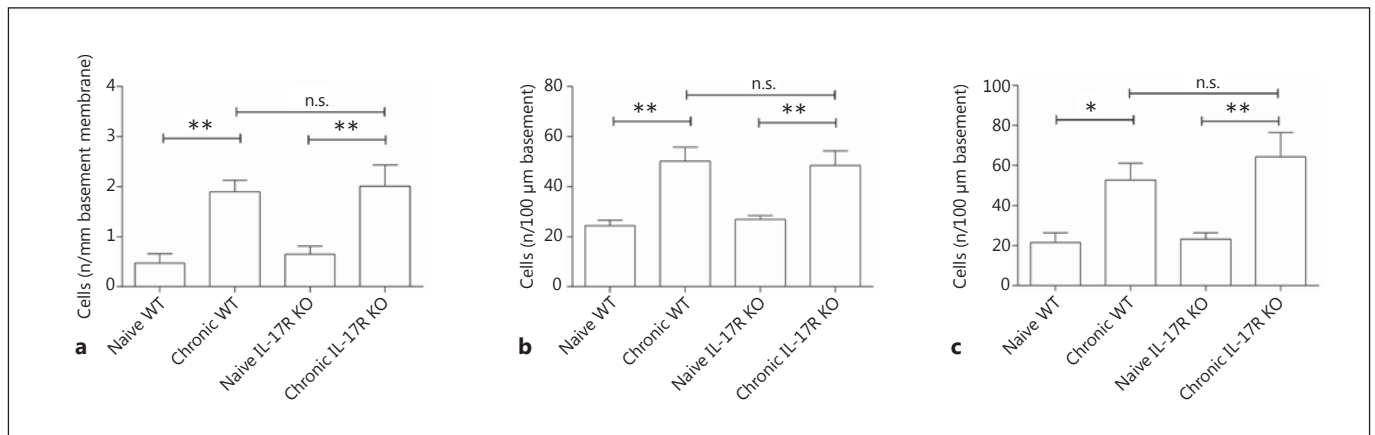


Fig. 5. Assessment of airway inflammation in IL-17R-deficient animals. Morphometric quantification of the profile density of intra-epithelial eosinophils (**a**), chronic inflammatory cells in the lamina propria of the trachea (**b**), and Gr-1-positive neutrophils

(**c**) in lung tissue, after 8 weeks of chronic challenge. Values are expressed as means $\pm$ SEM ($n = 8$ animals per group). Significant differences are shown as * $p < 0.05$ and ** $p < 0.01$. n.s. = Not significant.

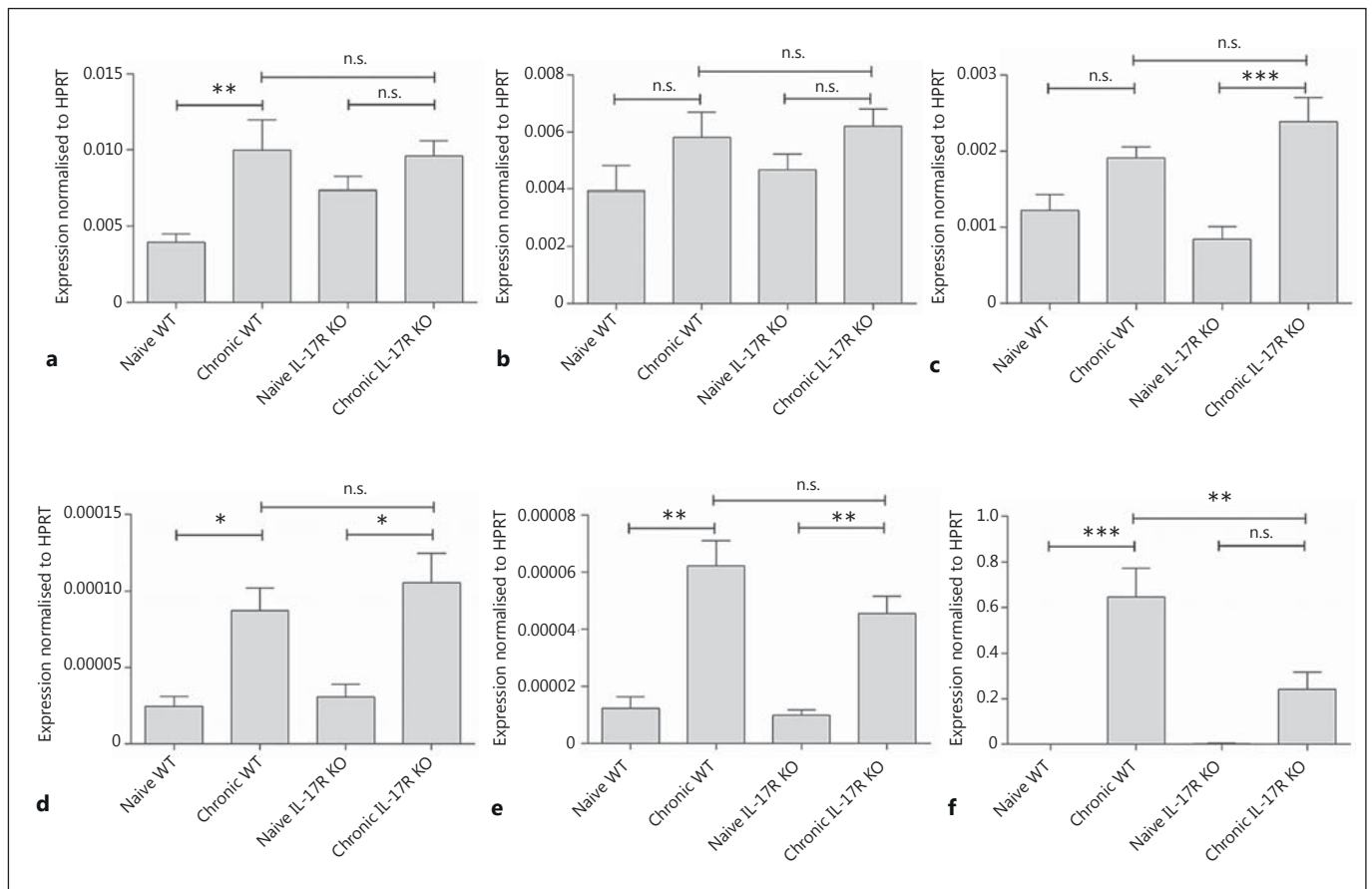


Fig. 6. Expression of mRNA in proximal airway tissues. Expression of mRNA for cytokines in proximal airway tissues of naive and sensitised, chronically challenged, WT and IL-17R KO mice normalised to HPRT. **a** CXCL1. **b** TNF- α . **c** IFN- γ . **d** IL-4. **e** IL-13

Gob-5. Data are means $\pm$ SEM ($n = 8$ animals per group). Significant differences compared to naive WT mice are shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. n.s. = Not significant.

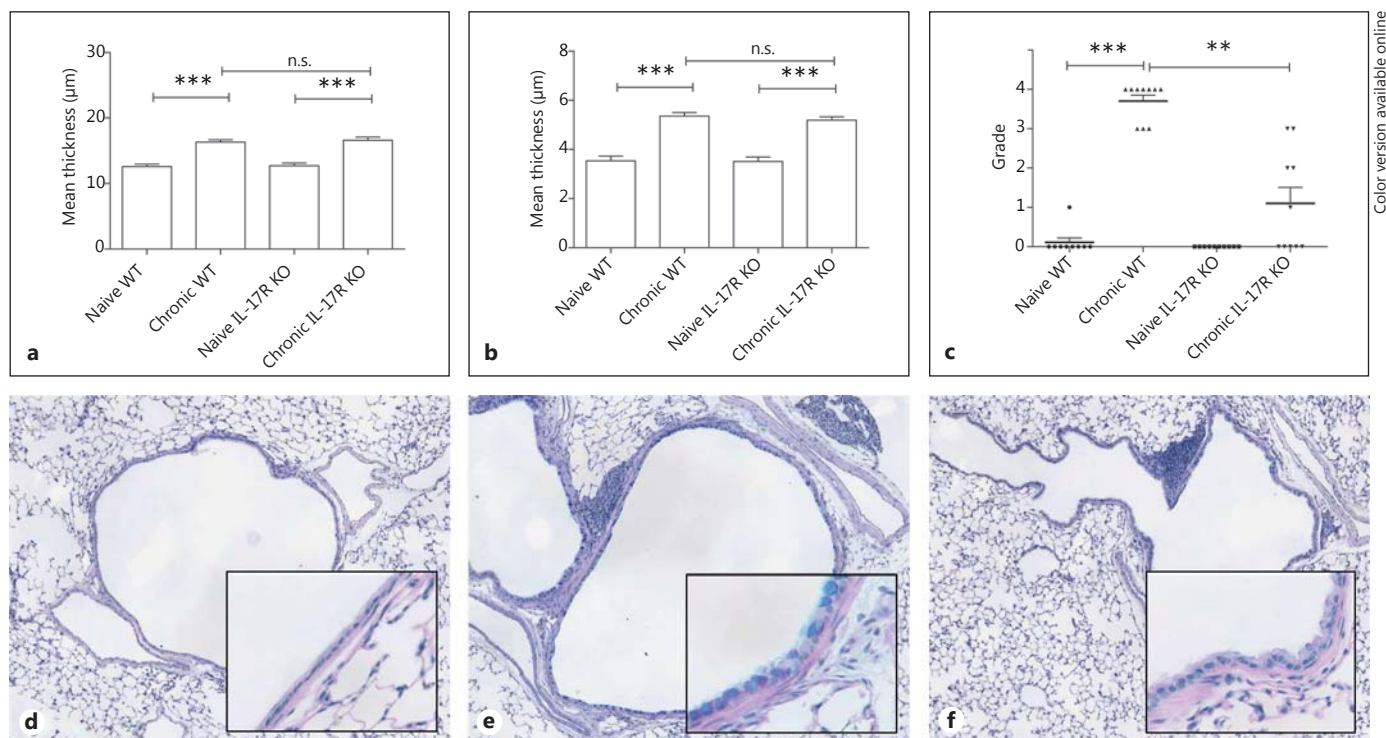


Fig. 7. Assessment of remodelling in IL-17R-deficient animals. Morphometric quantification of the thickness of the tracheal airway epithelium (**a**), sub-epithelial collagenisation in the trachea (**b**) and grade of mucous cell change in intra-pulmonary airways (**c**), after 8 weeks of chronic challenge. Values are expressed as means $\pm$ SEM (**a**, **b**) or medians $\pm$ IQR (**c**) ($n = 8$ animals per

group). Significant differences are shown as ** $p < 0.01$ and *** $p < 0.001$. Images of Alcian Blue/PAS-stained lung sections from naive (**d**), chronically challenged WT (**e**) and chronically challenged IL-17R KO (**f**) animals (original magnification $\times 100$). The **insets** show high-power images (original magnification $\times 400$). n.s. = Not significant.

challenge was strikingly absent in IL-17R-deficient animals (fig. 7c–f). This decreased response was paralleled by alterations in Gob-5 expression, which was significantly increased in WT but not in IL-17R-deficient animals (fig. 6).

Discussion

In these experiments, we have established that in our model of mild chronic asthma pulmonary CD4⁺ T cells exhibit increased production of Th17 cytokines. This finding is consistent with our previous observation of increased expression of IL-17A in the airways in this model [21] and indicates that CD4⁺ cells participating in the inflammatory response have differentiated towards a Th17 phenotype.

The results of our in vitro studies are consistent with reports suggesting that IL-17A induces production of

pro-inflammatory cytokines by AEC and fibroblasts [12, 14, 25, 26]. They are also consistent with published data indicating that IL-17A and Th17 cells enhance production of pro-remodelling cytokines by AEC and fibroblastic cells [17, 27]. However, whereas it has previously been reported that IL-17A stimulates the mucin genes MUC5AC and MUC5B [28], we were unable to confirm a significant effect on mucous differentiation in our culture system.

While our results support the concept that there may be a significant interaction between IL-17A and structural cells [29], our comparison of the cellular responses to conditioned media from Th1, Th2 and Th17 cells emphasises that Th2 cells have an effect similar to that of Th17 cells with respect to driving cytokine production by AEC. Thus our data strongly suggest that any selective effect of Th17 cytokines on airway inflammation and remodelling is likely to be via stimulation of fibroblasts rather than AEC.

Importantly, our experiments using IL-17R-deficient mice indicate that signalling via IL-17R does not play a significant role in inflammation associated with mild chronic asthma. This is in contrast to neutrophilic asthma or severe steroid-resistant asthma [30], in which IL-17A appears to have a role in the recruitment of neutrophils, which could be via the enhancement of TNF- α induced inflammation [31]. Furthermore, although IL-17A has been shown to stimulate the production of CXC chemokines [32, 33], we did not observe any effect on the expression of CXCL1.

Our results do indicate that IL-17R signalling contributes to airway remodelling, but in this model of mild chronic asthma we found that this pathway made only a limited contribution to the development of characteristic lesions. Thus, IL-17R-deficient mice developed epithelial thickening and sub-epithelial fibrosis similar to WT animals. However, these mice did exhibit significantly fewer mucus-producing cells in the airways, consistent with other recent studies [34].

Our results are in contrast to the observations of Wang et al. [17] who used a high-level chronic challenge model in C57BL/6 mice to assess the effects of inhibiting IL-17A signalling by administration of a neutralising monoclonal antibody. Those authors found not only that IL-17A had an essential role in the mucous cell response but also that, via signalling through the EGF receptor, it was involved in fibrosis and thickening of airway smooth muscle. However, in their model, the uncontrolled exposure to aero-

solised OVA led to a much more marked inflammatory response and much greater collagen accumulation, which was peribronchiolar rather than sub-epithelial. The different pattern of lesions is a feature of all such high-level challenge models and is quite dissimilar to that in human asthma [35]. In our more realistic model, we found that IL-17R signalling made only a limited contribution to the development of characteristic lesions of asthma. It is possible that other cytokines produced by Th17 cells, including IL-21 and IL-22, may have additional roles in the development of these lesions.

In conclusion, we have demonstrated that even though IL-17A and Th17 cells stimulate the production of pro-inflammatory and pro-remodelling cytokines by structural cells of the airways in vitro, abrogation of IL-17R signalling does not significantly attenuate the development of airway inflammation in a model of mild chronic asthma. Furthermore, the effect on airway remodelling is limited to regulation of the mucous cell response. Thus, targeting cytokines of the IL-17 family may be of limited value in reducing airway inflammation but could limit excessive production of mucus in patients with asthma.

Acknowledgements

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