

Effects of Anticytokine Therapy in a Mouse Model of Chronic Asthma

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The relative contribution of Th2 and Th1 cytokines to the pathogenesis of lesions of chronic asthma remains poorly understood. To date, therapeutic inhibition of Th2 cytokines has proved disappointing. We used a clinically relevant model of chronic allergic asthma in mice to compare the effects of administering neutralizing antibodies to interleukin (IL)-13, IL-5, and interferon- γ (IFN- γ) to animals with established disease. As has been observed in clinical studies, anti-IL-5 inhibited both inflammation and remodeling but had no effect on airway responsiveness to methacholine. Anti-IL-13 effectively suppressed eosinophil recruitment and accumulation of chronic inflammatory cells in the airways. This treatment also partially suppressed changes of airway wall remodeling, including goblet cell hyperplasia/metaplasia and subepithelial fibrosis, but had limited ability to inhibit airway hyperreactivity (AHR). In contrast, treatment with anti-IFN- γ markedly suppressed AHR. This antibody inhibited accumulation of chronic inflammatory cells but did not affect eosinophil recruitment or changes of remodeling. We conclude that inhibition of IL-5 is beneficial and that inhibition of IL-13 has considerable potential as a therapeutic strategy in chronic asthma, that IFN- γ may play an important role in the pathogenesis of AHR, and that co-operative interaction between Th2 and Th1 cytokines contributes to the pathogenesis of the lesions of chronic asthma.

Keywords: airway remodeling; bronchial hyperreactivity; interferon- γ ; interleukin-5; interleukin-13

Allergic asthma is characterized by episodes of acute inflammation of the airways superimposed on a background of chronic inflammation, structural changes collectively referred to as airway wall remodeling, and airway hyperreactivity (AHR) to a variety of stimuli (1). The immunologic response is dominated by CD4⁺ T cells, which exhibit a so-called Th2 profile of cytokine secretion, biased toward production of interleukin (IL)-4, IL-5, IL-9, and IL-13 (2). Studies in animal models of allergic bronchopulmonary inflammation, including investigations in gene-targeted mice deficient in one or more Th2 cytokines, have suggested that the manifestations of asthma may be ameliorated by targeting these mediators (3–11). However, clinical trials involving anti-IL-5 or soluble IL-4 receptor have thus far proved to be disappointing (12–14). This discordance may be related to the failure of animal models involving short-term challenge with

high levels of antigen to replicate many of the features of chronic human asthma (15) or to the induction of various compensatory mechanisms in gene-targeted cytokine-deficient animals (16, 17).

Reliable assessment of potential treatments is more likely to be achieved using an experimental model of chronic allergic disease of the airways. We have described such a model in mice that are systemically sensitized to ovalbumin and subjected to inhalational challenge with controlled low levels of aerosolized antigen for 6–8 weeks. This elicits lesions typical of chronic human asthma, including recruitment of eosinophils into the airway epithelium, chronic inflammation in the lamina propria, subepithelial fibrosis, and mucous cell hyperplasia/metaplasia. The exposure protocol is not associated with development of inflammation in the lung parenchyma but instead elicits recruitment of fewer total inflammatory cells and development of lesions confined to the conducting airways (18). In addition, the mice exhibit marked hyperreactivity to an inhaled cholinergic agonist, which is demonstrably of airway rather than parenchymal origin (19). Both the inflammatory response and AHR are CD4⁺ T-cell dependent (20), and there is evidence of a Th2 pattern of local immune response (21).

In this study, we used this model to examine the effect of administering neutralizing anticytokine antibodies to animals with established airway disease. There is currently considerable interest in IL-13 as a potential therapeutic target because in animal models of allergic inflammation it appears to be important in the induction of airway inflammation, subepithelial fibrosis, and AHR (10, 22–27). We therefore sought to compare anti-IL-13 with anti-IL-5. In addition, because we have previously demonstrated that Th1 cytokines such as interferon- γ (IFN- γ) may contribute to the pathogenesis of lesions in chronic asthma in this model (21), we examined the effect of anti-IFN- γ . We specifically wished to assess the contribution of each of these cytokines to airway inflammation, remodeling, and AHR in chronic asthma, as well as the relationship between these abnormalities, as revealed after therapeutic suppression of cytokine activity.

METHODS

Additional detail about these methods is provided in the online supplement.

Experimental Model

The protocols we employed for sensitization and inhalational challenge of mice have previously been described in detail (18, 28). Briefly, specific pathogen-free female BALB/c mice (aged approximately 8 weeks at the commencement of experimental studies) received intraperitoneal injections of 50 μ g of alum-precipitated chicken egg ovalbumin (Sigma Australia, Sydney, Australia) 21 days and 7 days before inhalational exposure. The animals were challenged with aerosolized ovalbumin for 30 minutes/day on 3 days/week for 6 weeks in a whole-body inhalation exposure chamber (Unifab Corporation, Kalamazoo, MI). Particle concentration within the breathing zone of the mice was continuously monitored and maintained at approximately equal to 3 mg/m³, as assessed by a real-time monitor (DustTrak 8520; TSI, St. Paul, MN).

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Experimental groups each comprised eight animals. All experimental procedures complied with the requirements of the Animal Care and Ethics Committee of the University of New South Wales (reference no. 01/34.1).

Treatment with Neutralizing Anticytokine Antibodies

During the last 2 weeks of antigen challenge (Days 29, 34, 38, and 42 of exposure), mice were administered intraperitoneal injections of protein G-purified rat anti-mouse-IL-13 antibody (C531; Centocor Inc., Malvern, PA; 0.5 mg per injection) or of ammonium sulfate-purified rat anti-mouse-IL-5 monoclonal antibody (TRFK-5, 0.5 mg per injection) (29), anti-mouse IFN- γ (R4-6A2, a generous gift from Dr. James Beck, University of Michigan, 0.35 mg per injection), or isotype control monoclonal antibody (β GL-113, 0.5 mg per injection). Antibody doses were based on published studies (9, 30–32). Untreated, nonsensitized, nonexposed (i.e., naïve) animals were assessed in parallel.

Histopathology and Morphometry

The approach to histopathologic examination as well as the validity and reliability of the morphometric techniques we employed for quantifying inflammation and remodeling has been documented in previous reports (18, 27, 28). Although changes of inflammation and remodeling are demonstrable in the trachea, main bronchi, and intrapulmonary airways in this model, for convenience, changes were quantified in sections of the longitudinally oriented trachea. Eosinophils within the airway epithelial layer and total numbers of nuclei in the lamina propria were counted in hematoxylin and eosin-stained sections. The thickness of the subepithelial zone of collagenization was measured in reticulin-stained sections. Mucus-secreting goblet cells in intrapulmonary airways were quantified according to a semilogarithmic grading scale in sections stained with Alcian blue-periodic acid-Schiff.

Airway Reactivity

Responsiveness to methacholine (aerosolized from solutions containing 3.125 to 50 mg/ml) was assessed as enhanced Pause (Penh), measured in conscious, unrestrained mice using apparatus and software supplied by Buxco (Troy, NY). Measurement was performed as previously described (28).

Statistical Analysis

Results of morphometry and assessment of airway reactivity are presented as arithmetic mean \pm SEM for each experimental group. Analysis of variance followed by Newman-Keuls multiple comparison test was used to examine differences between groups. The proportions of mucin-positive cells in the intrapulmonary airways were assigned grades and are expressed as median values, with comparisons undertaken using a nonparametric Kruskal-Wallis test followed by Dunn's test. The software package GraphPad Prism 4.01 (GraphPad Software, San Diego, CA) was used for all data analysis and preparation of graphs.

RESULTS

No adverse effects of intraperitoneal injection of antibodies were observed in any of the experimental groups studied.

Airway Inflammation

Whereas eosinophils are rarely identifiable within the tracheal epithelium of normal BALB/c mice, sensitized mice treated with control antibody β GL-113 exhibited recruitment of numerous intraepithelial eosinophils ($p < 0.001$ compared with nonexposed control animals; Figure 1). Treatment with anti-IL-13 markedly reduced the accumulation of eosinophils ($p < 0.01$ compared with β GL-113-treated control animals) as did treatment with anti-IL-5 ($p < 0.01$). In contrast, treatment with anti-IFN- γ had no effect on eosinophil recruitment.

Sensitized chronically exposed BALB/c mice treated with the control antibody developed widespread multifocal accumulation of lymphocytes, plasma cells, and other chronic inflammatory cells in the lamina propria of the trachea, leading to a marked increase in the number of cells in this compartment (Figure 2)

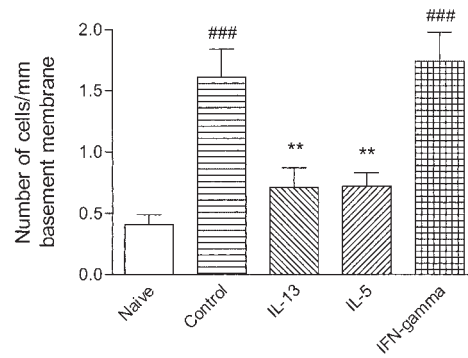


Figure 1. Accumulation of eosinophils in the tracheal epithelium of sensitized, chronically challenged mice treated with neutralizing anticytokine antibodies. Significant differences compared with naive animals shown as ### $p < 0.001$; significant differences compared with mice treated with the control antibody shown as ** $p < 0.01$. IFN = interferon; IL = interleukin.

($p < 0.001$ compared with nonexposed control animals). Accumulation of lamina propria cells was significantly diminished in mice treated with anti-IL-13 or anti-IFN- γ ($p < 0.01$ for both) and was also reduced in mice treated with anti-IL-5 ($p < 0.05$).

Airway Wall Remodeling

Mucus-secreting goblet cells, which are virtually absent in the intrapulmonary airways of naïve mice, were strikingly increased in sensitized exposed mice treated with the control β GL-113 antibody, with a median grade of 4 (Figure 3) ($p < 0.001$ compared with nonexposed control animals). In mice treated with anti-IL-13, there were substantially fewer mucous cells, with a median grade of 2. This was significantly decreased compared with β GL-113-treated control animals ($p < 0.05$) but was no longer significantly greater than nonexposed control animals. Treatment with anti-IL-5 led to a modest reduction in the proportion of goblet cells in the airways, but this was not statistically significant. Treatment with anti-IFN- γ had no significant effect on the number of mucous cells.

Sensitized mice exposed to antigen and treated with the control antibody exhibited accumulation of subepithelial collagen (Figure 4), leading to significant thickening of the reticulin-stained

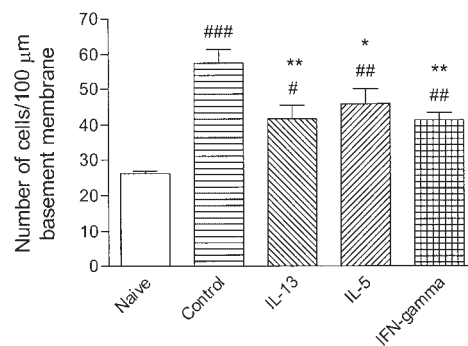


Figure 2. Inflammatory cells in the lamina propria of the trachea of sensitized, chronically challenged mice treated with neutralizing anticytokine antibodies. Significant differences compared with naive animals shown as # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$; significant differences compared with mice treated with the control antibody shown as * $p < 0.05$, ** $p < 0.01$.

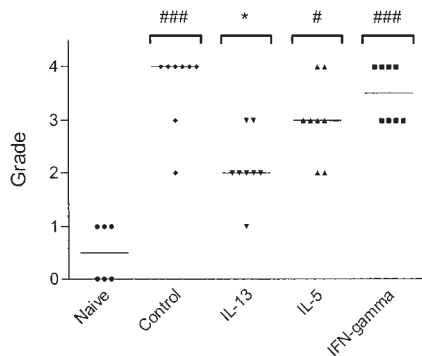


Figure 3. Mucous cell hyperplasia/metaplasia in the intrapulmonary airways of sensitized, chronically challenged mice treated with neutralizing anticytokine antibodies. Significant differences compared with naive animals shown as # $p < 0.05$, ### $p < 0.001$; significant difference compared with mice treated with the control antibody shown as * $p < 0.05$.

zone ($p < 0.001$ compared with nonexposed control animals). There was diminished subepithelial fibrosis in mice treated with anti-IL-13 or with anti-IL-5, although only the latter was statistically significant ($p < 0.05$) (Figure 4). Treatment with anti-IFN- γ had no effect on subepithelial fibrosis.

Airway Responsiveness

Animals treated with control β GL-113 antibody exhibited a left-shifted Penh dose-response curve and increased maximal reactivity to methacholine, characteristic of AHR (Figure 5). Treatment with anti-IL-13 led to a modest decrease in airway responsiveness, but this was statistically significant only at a methacholine concentration of 50 mg/ml. Anti-IL-5 had virtually no effect on airway responsiveness. In contrast, animals treated with anti-IFN- γ demonstrated statistically significant reductions in Penh at all methacholine concentrations above 3.125 mg/ml (Figure 5).

DISCUSSION

In this study, we employed a clinically relevant experimental model that replicates most of the features of chronic human asthma with high fidelity (15), to assess the effect of treatment with neutralizing antibodies against IL-13, IL-5, and IFN- γ . We

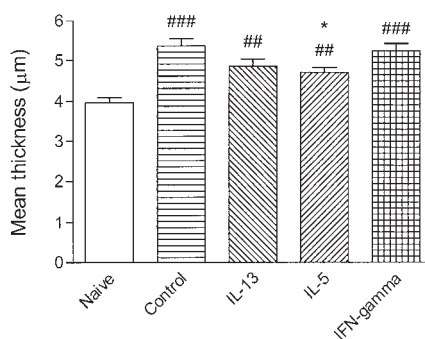


Figure 4. Subepithelial collagenization in the trachea of sensitized, chronically challenged mice treated with neutralizing anticytokine antibodies. Significant differences compared with naive animals shown as ## $p < 0.01$, ### $p < 0.001$; significant difference compared with mice treated with the control antibody shown as * $p < 0.05$.

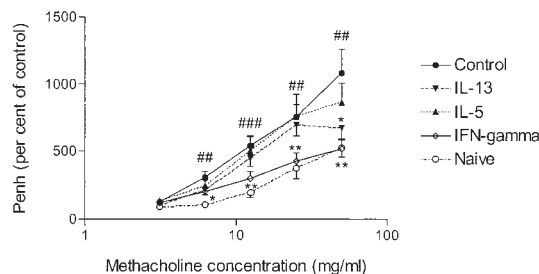


Figure 5. Airway reactivity of sensitized, chronically challenged mice treated with neutralizing anticytokine antibodies, assessed by change in Penh in response to increasing concentrations of aerosolized β -methacholine. Significant differences compared with naive animals shown as ## $p < 0.01$, ### $p < 0.001$; significant differences compared with mice treated with the control antibody shown as * $p < 0.05$, ** $p < 0.01$.

have previously shown that in this model, changes of inflammation and airway wall remodeling are established by 4 weeks and progress with continuing antigenic challenge (18). The therapeutic regimen was therefore also clinically relevant and allowed us to examine whether the antibodies could inhibit progression of lesions and/or reverse them. The three antibodies had strikingly different effects in this model of chronic asthma.

Treatment with anti-IL-5 had significant antiinflammatory effects, decreasing the numbers of both intraepithelial eosinophils and chronic inflammatory cells in the airway wall, yet failed to inhibit AHR. These observations closely parallel the findings in clinical trials in which humanized monoclonal antibodies were administered to subjects with asthma (12, 13). The data, which are in contrast to the findings in a short-term model in which there was partial inhibition of AHR (8), reinforce the similarity between our chronic challenge model and the human disease. However, the lack of inhibition of AHR by treatment with anti-IL-5 differs from our earlier findings in IL-5 $^{-/-}$ mice (28), which might be a consequence of associated cytokine disturbances in the gene-targeted animals; for example, there is evidence of deficient production of IL-13 in IL-5 $^{-/-}$ mice (33). Anti-IL-5 partially inhibited the mucous cell response, as others have previously reported in IL-5 $^{-/-}$ mice (34). In addition, administration of the antibody significantly inhibited the development of subepithelial fibrosis. This result is consistent with an earlier study in mice (30) and directly supports recent observations in humans (35), indicating that IL-5 plays a role in the development of airway wall remodeling.

Anti-IL-13 was also a potent antiinflammatory agent, significantly reducing the accumulation of intraepithelial eosinophils and of chronic inflammatory cells in the lamina propria of the airways. In addition, anti-IL-13 effectively inhibited mucous cell hyperplasia/metaplasia and caused a diminution in thickness of the reticulin-stained zone, although the latter response did not achieve statistical significance in a multiple comparison. Mice treated with anti-IL-13 still had evidence of AHR, but there was significant inhibition at the highest concentration of methacholine tested. Whereas studies in IL-13 $^{-/-}$ mice have yielded conflicting data (9, 10, 27), these results clearly demonstrate that treatment with anti-IL-13 had a combination of beneficial effects.

Our findings emphasize the potential of inhibition of IL-13 as a therapeutic strategy in chronic asthma. They are also consistent with data from two previously reported studies in models of chronic asthma, in which treatment with anti-IL-13 significantly inhibited eosinophil recruitment (32), collagen deposition in the

lungs, and mucous cell hyperplasia/metaplasia (26, 32). However, the results of those studies are not directly comparable, as both models were associated with marked parenchymal inflammation progressing to pulmonary fibrosis, which may impact interpretation of the data. The presence of parenchymal disease also makes it difficult to draw direct comparisons between the modest effect on AHR that we observed after administration of anti-IL-13 and the partial (26) or complete (32) inhibition of AHR demonstrated in those models.

In our model of chronic asthma, we have previously demonstrated virtually complete absence of subepithelial fibrosis in IL-13^{-/-} mice (27). The lesser effect after treatment with anti-IL-13 might be related to the dosage of antibody employed or the duration for which it was administered, although in previous experiments, we have shown that glucocorticoids or other anti-inflammatory agents could inhibit changes of remodeling when administered over a similar period (36).

The effects of anti-IFN- γ were in marked contrast to the other two neutralizing antibodies. Although anti-IFN- γ had no effect on eosinophil accumulation, it significantly diminished the accumulation of chronic inflammatory cells in the lamina propria. It had no apparent effect on remodeling, but very effectively suppressed AHR in our model of chronic asthma. This was an unexpected finding but is consistent with numerous published reports implying a role for Th1 cytokines and especially for IFN- γ in the pathogenesis of AHR, both in patients with asthma (37–41) and in animal models (42–44). IFN- γ may be particularly important in the development of AHR in models of chronic asthma, which involve distinctly different pathogenetic mechanisms as compared with short-term models of allergic bronchopulmonary inflammation. This has been well demonstrated in a model of fungal asthma (45) and in our model of chronic aerosol challenge (21): in both of these models, AHR is demonstrable in signal transducer and activator of transcription (STAT)6-deficient animals (which exhibit impaired Th2 cytokine signaling), whereas this deficiency abrogates AHR in short-term challenge models. We have also shown that in our model, AHR is regulated independently of airway inflammation or remodeling and can develop in animals deficient in IL-4, IL-13, or signaling via the IL-4 receptor α chain (reviewed in 15), which may be relevant to the observed pattern of response to antibody treatment. However, the molecular mechanisms regulating AHR clearly differ in other murine models of chronic allergen challenge. For example, models involving high-dose chronic aerosol exposure frequently exhibit downregulation of AHR (46), which has been demonstrated to be associated with a shift to a Th1 profile and induction of IFN- γ (47). In contrast, models involving chronic intranasal challenge usually exhibit persistent AHR (48, 49), which is abrogated in mice deficient in either IL-4 or IL-13 (50).

This study provides the first direct demonstration of the potential importance of IFN- γ in AHR in chronic asthma. In interpreting this finding, consideration needs to be given to the method we employed to assess airway responsiveness. Measurement of Penh by unrestrained plethysmography does not provide direct assessment of a specific physiologic variable, and its limitations are well documented (51–53). However, Penh can be used to monitor airway function empirically. In our chronic challenge model, we have previously shown that the observed changes in Penh closely correlate with increased specific airway resistance as measured by the forced oscillation technique and that the animals exhibit hyperreactivity, which originates from the airways and not the pulmonary parenchyma (19).

Although the cellular source of the IFN- γ that contributes to AHR in our model has not yet been defined, the absence of AHR in mice depleted of CD4⁺ T lymphocytes (20) suggests

that these cells may either be the source of IFN- γ or may regulate other cells producing this cytokine. In human asthma, both CD4⁺ and CD8⁺ T cells are important sources of IFN- γ (38, 54, 55). That production of IFN- γ by chronic inflammatory cells might play a key role in AHR provides a logical explanation for some of the striking differences in airway reactivity between acute and chronic antigenic challenge models (27, 28, 45). Investigators have previously suggested that IFN- γ may contribute to airway hyperresponsiveness by modulating the contractile responses of airway smooth muscle cells to leukotrienes (56). Treatment with anti-IFN- γ , which has long been recognized to inhibit lymphocyte accumulation at the site of an immunologic response (57), might also contribute to suppression of AHR by depletion of a relevant population of chronic inflammatory cells and/or their mediators.

In the context of our previously published studies in this model, the results of this study strongly support the notion that the pathogenesis of the lesions of asthma, and especially of AHR, involves a cooperative interaction between Th2 and Th1 cytokines (21, 58). Although targeting single cytokines or signaling pathways in therapy is unlikely to effectively control all of the manifestations of the disease, our observations suggest that inhibition of IL-5 may be beneficial in chronic asthma, that inhibition of IL-13 is a potentially useful therapeutic strategy, and that consideration should be given to inhibiting IFN- γ in parallel as an approach to combination therapy.

Conflict of Interest Statement: R.K.K. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; C.H. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; D.C.W. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript; L.L. is an employee of Centocor, Inc. (a Johnson & Johnson company) and holds Johnson & Johnson stock options; P.S.F. does not have a financial relationship with a commercial entity that has an interest in the subject of this manuscript. Centocor is interested in the therapeutic area in asthma.

References

1. Kumar RK. Understanding airway wall remodeling in asthma: a basis for improvements in therapy? *Pharmacol Ther* 2001;91:93–104.
2. Humbert M, Menz G, Ying S, Corrigan CJ, Robinson DS, Durham SR, Kay AB. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 1999;20:528–533.
3. Lukacs NW, Strieter RM, Chensue SW, Kunkel SL. Interleukin-4-dependent pulmonary eosinophil infiltration in a murine model of asthma. *Am J Respir Cell Mol Biol* 1994;10:526–532.
4. Mauer PJ, Pitman AM, Fernandez X, Foran SK, Adams GK, Kreutner W, Egan RW, Chapman RW. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care Med* 1995;152:467–472.
5. Brusselle G, Kips J, Joos J, Bluethmann H, Pauwels R. Allergen-induced airway inflammation and bronchial responsiveness in wild-type and interleukin-4-deficient mice. *Am J Respir Cell Mol Biol* 1995;12:254–259.
6. 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.
7. Hamelmann E, Oshiba A, Loader J, Larsen GJ, Gleich G, Lee J, Gelfand EW. Antiinterleukin-5 antibody prevents airway hyperresponsiveness in a murine model of airway sensitization. *Am J Respir Crit Care Med* 1997;155:819–825.
8. Hogan SP, Koskinen A, Foster PS. Interleukin-5 and eosinophils induce airway damage and bronchial hyperreactivity during allergic airway inflammation in BALB/c mice. *Immunol Cell Biol* 1997;75:284–288.
9. 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;165:108–113.
10. Walter DM, McIntire JJ, Berry G, McKenzie AN, Donaldson DD, De Kruff RH, Umetsu DT. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. *J Immunol* 2001;167:4668–4675.

11. Foster PS, Hogan SP, Yang M, Mattes J, Young IG, Matthaei KI, Kumar RK, Mahalingam S, Webb DC. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;8:162–167.
12. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144–2148.
13. Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HA, Postma DS, Danzig M, Cuss F, Pauwels RA. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655–1659.
14. Barnes PJ. Cytokine-directed therapies for the treatment of chronic airway diseases. *Cytokine Growth Factor Rev* 2003;14:511–522.
15. Kumar RK, Foster PS. Modeling allergic asthma in mice: pitfalls and opportunities. *Am J Respir Cell Mol Biol* 2002;27:267–272.
16. McKenzie GJ, Fallon PG, Emson CL, Grecis RK, McKenzie AN. Simultaneous disruption of interleukin (IL)-4 and IL-13 defines individual roles in T helper cell type 2-mediated responses. *J Exp Med* 1999;189:1565–1572.
17. McMillan SJ, Bishop B, Townsend MJ, McKenzie AN, Lloyd CM. The absence of interleukin 9 does not affect the development of allergen-induced pulmonary inflammation nor airway hyperreactivity. *J Exp Med* 2002;195:51–57.
18. 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;53:849–856.
19. Collins RA, Sly PD, Turner DJ, Herbert C, Kumar RK. Site of inflammation influences site of hyperresponsiveness in experimental asthma. *Respir Physiol Neurobiol* 2003;139:51–61.
20. Foster PS, Yang M, Herbert C, Kumar RK. CD4+ T-lymphocytes regulate airway remodeling and hyper-reactivity in a mouse model of chronic asthma. *Lab Invest* 2002;82:455–462.
21. Foster PS, Webb DC, Yang M, Herbert C, Kumar RK. Dissociation of T helper type 2 cytokine-dependent airway lesions from signal transducer and activator of transcription 6 signalling in experimental chronic asthma. *Clin Exp Allergy* 2003;33:688–695.
22. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, Donaldson DD. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258–2261.
23. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, Sheppard D, Mohrs M, Donaldson DD, Locksley RM, *et al.* Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261–2263.
24. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 1999;103:779–788.
25. Yang M, Hogan SP, Henry PJ, Matthaei KI, McKenzie AN, Young IG, Rothenberg ME, Foster PS. Interleukin-13 mediates airways hyper-reactivity through the IL-4 receptor- α chain and STAT-6 independently of IL-5 and eotaxin. *Am J Respir Cell Mol Biol* 2001;25:522–530.
26. Blease K, Jakubzick C, Westwick J, Lukacs N, Kunkel SL, Hogaboam CM. Therapeutic effect of IL-13 immunoneutralization during chronic experimental fungal asthma. *J Immunol* 2001;166:5219–5224.
27. Kumar RK, Herbert C, Yang M, Koskinen AML, McKenzie ANJ, Foster PS. Role of interleukin-13 in eosinophil accumulation and airway remodelling in a mouse model of chronic asthma. *Clin Exp Allergy* 2002;32:1104–1111.
28. Foster PS, Ming Y, Matthaei KI, Young IG, Temelkovski J, Kumar RK. Dissociation of inflammatory and epithelial responses in a murine model of chronic asthma. *Lab Invest* 2000;80:655–662.
29. Hogan SP, Matthaei KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity in BALB/c mice independently of IL-4 and IL-5. *J Immunol* 1998;161:1501–1509.
30. Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell Mol Biol* 2000;23:241–246.
31. Garry BA, Gigliotti F, Harmsen AG. Neutralization of interferon- γ exacerbates pneumocystis-driven interstitial pneumonitis after bone marrow transplantation in mice. *J Clin Invest* 1997;99:1637–1644.
32. Li L, Yang G, Griswold DE, Shealy D, Emmell E, Shang XZ, Li J, Volk A. Anti-IL-13 monoclonal antibody inhibits airway response, inflammation and airway remodeling [abstract]. *Am J Respir Crit Care Med* 2003;167:A955.
33. Mattes J, Yang M, Mahalingam S, Kuehr J, Webb DC, Simson L, Hogan SP, Koskinen A, McKenzie ANJ, Dent LA, *et al.* Intrinsic defect in T cell production of interleukin (IL)-13 in the absence of both IL-5 and eotaxin precludes the development of eosinophilia and airways hyperreactivity in experimental asthma. *J Exp Med* 2002;195:1433–1444.
34. Trifillieff A, Fujitani Y, Coyle AJ, Kopf M, Bertrand C. IL-5 deficiency abolishes aspects of airway remodelling in a murine model of lung inflammation. *Clin Exp Allergy* 2001;31:934–942.
35. Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, Barnes N, Robinson D, Kay AB. Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029–1036.
36. Kumar RK, Herbert C, Thomas PS, Wollin L, Beume R, Yang M, Webb DC, Foster PS. Inhibition of inflammation and remodeling by roflumilast and dexamethasone in murine chronic asthma. *J Pharmacol Exp Ther* 2003;307:349–355.
37. Krug N, Madden J, Redington AE, Lackie P, Djukanovic R, Schauer U, Holgate ST, Frew AJ, Howarth PH. T-cell cytokine profile evaluated at the single cell level in BAL and blood in allergic asthma. *Am J Respir Cell Mol Biol* 1996;14:319–326.
38. Magnan AO, Mely LG, Camilla CA, Badier MM, Montero-Julian FA, Guillot CM, Casano BB, Prato SJ, Fert V, Bongrand P, *et al.* Assessment of the Th1/Th2 paradigm in whole blood in atopy and asthma: increased IFN- γ -producing CD8+ T cells in asthma. *Am J Respir Crit Care Med* 2000;161:1790–1796.
39. Busse WW, Lemanske RF. Asthma. *N Engl J Med* 2001;344:350–362.
40. Koh YI, Choi IS, Lee HC. Relationship between changes in interferon- γ production by peripheral blood T cells and changes in peak expiratory flow rate in patients with chronic stable asthma. *Clin Exp Allergy* 2002;32:1734–1738.
41. Brown V, Warke TJ, Shields MD, Ennis M. T cell cytokine profiles in childhood asthma. *Thorax* 2003;58:311–316.
42. Hessel EM, van Oosterhout AJM, van Ark I, van Esch B, Hofman G, van Loveren H, Savelkoul HFJ, Nijkamp FP. Development of airway hyperresponsiveness is dependent on interferon- γ and independent of eosinophil infiltration. *Am J Respir Cell Mol Biol* 1997;16:325–334.
43. Aronica MA, Mora AL, Mitchell DB, Finn PW, Johnson JE, Sheller JR, Boothby MR. Preferential role for NF- κ B/Rel signaling in the type 1 but not type 2 T cell-dependent immune response in vivo. *J Immunol* 1999;163:5116–5124.
44. Fleming CM, He H, Ciota A, Perkins D, Finn PW. Administration of pentoxifylline during allergen sensitization dissociates pulmonary allergic inflammation from airway hyperresponsiveness. *J Immunol* 2001;167:1703–1711.
45. Blease K, Schuh JM, Jakubzick C, Lukacs NW, Kunkel SL, Joshi BH, Puri RK, Kaplan MH, Hogaboam CM. Stat6-deficient mice develop airway hyperresponsiveness and peribronchial fibrosis during chronic fungal asthma. *Am J Pathol* 2002;160:481–490.
46. Shore SA. Modeling airway remodeling: the winner by a nose? *Am J Respir Crit Care Med* 2003;168:910–911.
47. Cui ZH, Joetham A, Aydtintug MK, Hahn YS, Born WK, Gelfand EW. Reversal of allergic airway hyperreactivity after long-term allergen challenge depends on γ madelta T cells. *Am J Respir Crit Care Med* 2003;168:1324–1332.
48. Shinagawa K, Kojima M. Mouse model of airway remodeling: strain differences. *Am J Respir Crit Care Med* 2003;168:959–967.
49. Cho JY, Miller M, Baek KJ, Han JW, Nayyar J, Rodriguez M, Lee SY, McElwain K, McElwain S, Raz E, *et al.* Immunostimulatory DNA inhibits transforming growth factor- β expression and airway remodeling. *Am J Respir Cell Mol Biol* 2004;30:651–661.
50. Leigh R, Ellis R, Wattie JN, Hirota JA, Matthaei KI, Foster PS, O'Byrne PM, Inman MD. Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. *Am J Respir Crit Care Med* 2003;169:860–867.
51. Drazen JM, Finn PW, De Sanctis GT. Mouse models of airway responsiveness: physiological basis of observed outcomes and analysis of selected examples using these outcome indicators. *Annu Rev Physiol* 1999;61:593–625.
52. Lundblad LK, Irvin CG, Adler A, Bates JH. A reevaluation of the validity of unrestrained plethysmography in mice. *J Appl Physiol* 2002;93:1198–1207.

53. Adler A, Cieslewicz G, Irvin CG. Unrestrained plethysmography is an unreliable measure of airway responsiveness in BALB/c and C57BL/6 mice. *J Appl Physiol* 2004;97:286–292.
54. Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma. *Am Rev Respir Dis* 1990;141:970–977.
55. O'Sullivan S, Cormican L, Faul JL, Ichinohe S, Johnston SL, Burke CM, Poulter LW. Activated, cytotoxic CD8(+) T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 2001;164:560–564.
56. Amrani Y, Moore PE, Hoffman R, Shore SA, Panettieri RA Jr. Interferon-gamma modulates cysteinyl leukotriene receptor-1 expression and function in human airway myocytes. *Am J Respir Crit Care Med* 2001;164:2098–2101.
57. Issekutz TB, Stoltz JM, van der Meide P. Lymphocyte recruitment in delayed-type hypersensitivity: the role of IFN-gamma. *J Immunol* 1988;140:2989–2993.
58. Mattes J, Yang M, Siqueira A, Clark K, MacKenzie J, McKenzie ANJ, Webb DC, Matthaei KI, Foster PS. IL-13 induces airways hyperreactivity independently of the IL-4Ralpha chain in the allergic lung. *J Immunol* 2001;167:1683–1692.