

The trials and tribulations of IL-5, eosinophils, and allergic asthma

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Eosinophils have been suggested to be part of the pathologic process that characterizes asthma, and their recruitment into the upper or lower airways appears to be essential for the clinical manifestations of allergen inhalation. IL-5 is a cytokine necessary for the development, differentiation, recruitment, activation, and survival of eosinophils. Allergen inhalation increases the production of IL-5 in the airways as measured in bronchoalveolar lavage cells and induced sputum. The relationship between IL-5 and the development of airway eosinophilia has been firmly established in IL-5 transgenic mice, with allergen challenge models in IL-5-deficient mice, and in mice treated with blocking anti-IL-5 antibodies. In addition, an accumulation of evidence suggests that treating mice with anti-IL-5 blocking antibodies prevents allergen-induced airway hyperresponsiveness. A recently reported study examined the effects of treatment with a humanized anti-IL-5 mAb (SB-240563) on allergen-induced airway responses and inflammation in atopic subjects. The authors of the study concluded that their results call into question the role of eosinophils in mediating the allergen-induced late asthmatic response and airway hyperresponsiveness; however, because of methodologic limitations, the study cannot be used either to support or to refute the concept of an important role for eosinophils in causing allergen-induced changes in airway function. (*J Allergy Clin Immunol* 2001;108:503-8.)

Key words: Interleukin-5, eosinophils, allergen, asthma, airway hyperresponsiveness, late asthmatic responses

For more than 80 years, eosinophils have been suggested to be part of the pathologic process that characterizes asthma.¹ In that time, much work has been undertaken in an effort to understand the precise role that eosinophils, as well as the mediators of inflammation and tissue damage and repair that eosinophils produce, play in asthma pathogenesis. Indeed, the association of airway eosinophilic inflammation and symptomatic asthma is so strong that one prominent asthma researcher has suggested that asthma should be renamed "eosinophilic bronchi-

Abbreviations used

AHR: Airway hyperresponsiveness
Eo/B-CFU: Eosinophil/basophil colony-forming unit
hmAb: Humanized monoclonal antibody
IL-5KO: IL-5-deficient
IL-4KO: IL-4-deficient
PC₂₀: Provocative concentration causing a 20% fall in FEV₁

tis" to highlight the central role that eosinophils play in its pathogenesis.²

Since the identification of IL-5 in the 1980s,³ it has become increasingly clear that in contrast to other cytokines thought to be involved in allergic inflammation, such as GM-CSF and IL-3, the biological activity of IL-5 is very specifically focused on the development,⁴ differentiation,⁵ recruitment,⁶ activation,⁷ and survival⁸ of a single cell type, the eosinophil. This has stimulated great interest, inasmuch as increased eosinophil production and recruitment into the upper or lower airways appears to be essential for the clinical manifestations of allergen inhalation at these sites. The specificity of IL-5 has also raised the possibility that blocking its activity at its receptor or at a transcriptional level might be a useful therapy for allergic diseases.⁹

ALLERGEN INHALATION AS A CLINICAL MODEL

Allergen inhalation challenge has been a well-characterized clinical model by which to study the pathophysiology of allergen-induced effects on the upper and lower airways. Allergen inhalation into the lower airways by allergic subjects results in the development of acute bronchoconstriction, which is mediated mainly by cysteinyl leukotriene and histamine release¹⁰ (the early response), and the development, in more than half of subjects, of late bronchoconstrictor responses beginning 2 to 4 hours after inhalation and able to persist for more than 24 hours.¹¹ These bronchoconstrictor responses are again mediated mainly by cysteinyl leukotriene and histamine,¹⁰ likely released from inflammatory cells newly recruited into the airways. The cells recruited at the time of the late response are mainly eosinophils¹² and basophils.¹³ With respect to the time course of their recruitment, these inflammatory

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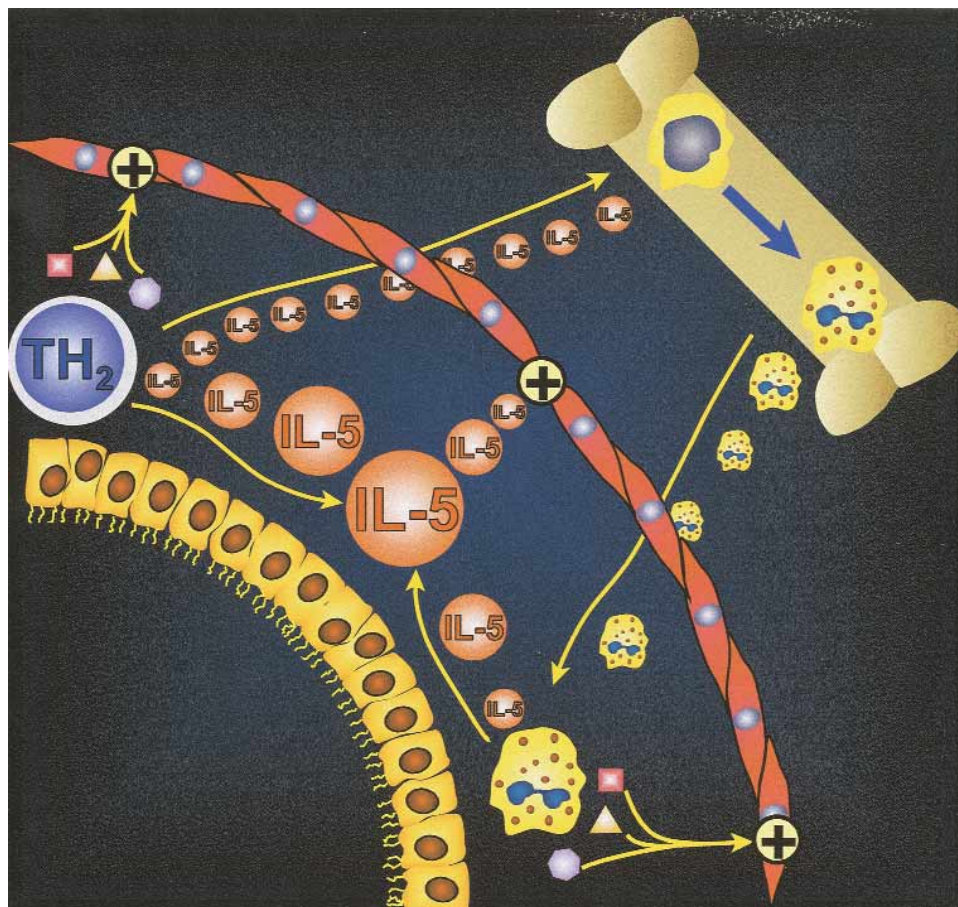


FIG 1. Schematic representation of the possible role of IL-5 in causing airway eosinophilia in asthma.

cells are different. Basophils appear to be maximal at 7 hours (the earliest time point evaluated) after inhaled allergen and are decreased by 24 hours,¹³ whereas eosinophils are maximal at 24 hours and take up to 1 week to return to baseline values.¹² The increases in eosinophils are temporally associated with the persistence of allergen-induced airway hyperresponsiveness (AHR).¹²

Allergen inhalation increases the production of IL-5 in the airways as measured in bronchoalveolar lavage cells¹⁴ and induced sputum.¹⁵ Interestingly, eosinophils are one of the cell types responsible for this increase.¹⁴ Furthermore, allergen inhalation increases the number of peripheral blood eosinophils and lymphocytes containing intracellular IL-5.¹⁶ The relatively high numbers of lymphocytes positive for IL-5 suggest that IL-5 is contained not only in CD4⁺ T_H2 cells but also in other lymphocytes, including CD8⁺ and CD4⁺CD8⁺ cells.

IL-5 also plays a critical role in eosinophilopoiesis in the bone marrow (Fig 1). Eosinophil/basophil colony-forming units (Eo/B-CFUs) and other bone marrow progenitors express the cell surface marker CD34. An important aspect of allergic inflammatory responses is the induction of increases in inflammatory cell progenitors, which contribute to disease through the continued production of inflammatory effector cells. Higher numbers of

both circulating Eo/B-CFUs and CD34⁺ hemopoietic progenitor cells are demonstrable in the blood of atopic subjects but not in the blood of normal subjects.¹⁷ In addition, the number of Eo/B-CFUs in the circulation of an asthmatic subject at the time of an acute exacerbation is significantly higher than the number after resolution of the exacerbation.¹⁸ Studies in atopic subjects have shown that there are fluctuating numbers of circulating Eo/B-CFUs during seasonal exposure to allergen¹⁹ and significantly higher numbers 24 hours after allergen inhalation.²⁰ Increases in bone marrow Eo/B-CFUs have also been demonstrated in allergic asthmatic subjects after allergen inhalation²¹; in this study, subjects who developed dual asthmatic responses and had significantly greater numbers of eosinophils in the airways had significantly greater numbers of bone marrow Eo/B-CFUs when the cells were incubated with a suboptimal concentration of IL-5 than subjects with isolated early asthmatic responses with fewer airway eosinophils. This indicates that after allergen challenge, the bone marrow of the dual responders is more responsive to IL-5, which might reflect either a specific induction of a population of more committed eosinophil/basophil progenitors or an upregulation of the IL-5 receptor on the surface of these cells. Sehmi et al²² demonstrated an increase in the proportion

of CD34⁺ cells expressing the α subunit of the IL-5 receptor after allergen challenge in dual but not isolated early responders. These results suggest that the responsiveness of the bone marrow to IL-5 after allergen challenge is a determinant of the magnitude of the eosinophilic responses to inhaled allergen and that this is related to the degree of the subsequent physiologic abnormalities.

STUDIES IN ANIMAL MODELS

The relationship between IL-5 and the development of airway eosinophilia has been firmly established both in IL-5 transgenic mice^{23,24} and with allergen challenge models in IL-5-deficient (IL-5KO) mice,²⁵ as well as in mice treated with blocking anti-IL-5 antibodies.²⁶ However, the role of IL-5 and/or the resulting eosinophilia in the development of physiologic abnormalities in mouse models of allergen-induced hyperresponsiveness has been much more difficult to elucidate. Early results suggesting that AHR was prevented in IL-5KO mice²⁴ but not in mice treated with anti-IL-5 antibody²⁶ were conflicting. Further studies suggested that treatment with anti-IL-5, though it was effective at reducing eosinophilia, was not sufficient to prevent AHR.^{27,28} Instead, the investigators were able to prevent AHR by knocking out T cells²⁸ or treating mice with a blocking anti-DC4⁺ antibody,²⁷ suggesting that a non-IL-5, T-cell derived mediator was responsible for allergen-induced AHR. Further support for this line of reasoning came from studies in which blocking IL-13 was able to prevent allergen-induced AHR with little²⁹ or no³⁰ effect on the eosinophil response. Separation between allergen-induced eosinophilia and AHR was also demonstrated when AHR was induced without eosinophilia in mice exposed to low concentrations of inhaled house dust mite.³¹ However, there has continued to be an accumulation of studies indicating that treating mice with anti-IL-5 blocking antibodies is sufficient to prevent allergen-induced AHR.³²⁻³⁴ This line of evidence is supported by studies demonstrating AHR in transgenic mice with overproduction of IL-5 in the airway.²⁴

Possible reasons for the discrepancies between these findings include differences in the mouse strain used, the dose of allergen used (and its delivery route), the method used to measure AHR, and the timing of the drug administration. However, different results cannot always be explained in terms of differences between laboratories. Treatment with the same anti-IL-5 blocking antibody was shown to prevent allergen-induced AHR in one study of wild type BALB/c mice³³ but not in another study of IL-4KO BALB/c mice³⁵ in which the same allergen challenge model and measurement techniques were used by the same investigators in the same laboratory. These examples are not intended to reflect a criticism of the methods used in any of the laboratories; rather, they are cited to illustrate the difficulty of trying to logically synthesize all of the available information. It seems likely that the extent of differences in protocol/mouse strain/measurement techniques/housing conditions introduced too many degrees of freedom to make such a syn-

thesis possible. Interestingly, in a recent study from Tournoy et al,³⁶ treatment with anti-IL-5 antibody was able to prevent house dust mite-induced AHR in lymphocyte-deficient mice that had received T cells from human donors. These studies of allergic airway inflammation in animal models suggest a central role for IL-5 in the increased production of eosinophils after allergen inhalation and perhaps also in their prolonged survival in the airways. No other important biological role has been identified thus far for IL-5, and the information collected about a possible role for IL-5 in allergic asthma has provided a strong rationale for studies in which the activity of IL-5 has been blocked and the degree of allergen-induced eosinophilic airway inflammation and associated physiological changes have been evaluated in human subjects to determine how important this cytokine is in causing allergic disease.

EFFECTS OF ANTI-IL-5 ON HUMAN ALLERGEN-INDUCED RESPONSES

In a study that has recently been reported, investigators examined the effects of treatment with a humanized anti-IL-5 monoclonal antibody (hmAb; SB-240563) on allergen-induced airway responses and inflammation in atopic subjects.³⁷ In this double-blinded, placebo-controlled, parallel-group, randomized investigation, Leckie et al³⁷ treated mildly allergic asthmatic subjects with 1 of 2 doses of anti-IL-5 (2.5 or 10 mg/kg, given as a single intravenous infusion) or with placebo. Twenty-four subjects were treated (8 in each of the treatment arms), and the study was conducted in 3 centers (2 in the United Kingdom and 1 in The Netherlands). The effects of treatment were examined on blood and airway eosinophils (measured in induced sputum) and on the responses to inhaled allergen challenge administered at 1 week and 4 weeks after the treatment. The study demonstrated that treatment with the anti-IL-5 mAb significantly reduced blood eosinophils and sputum eosinophils for at least 4 weeks but did not have any significant effect on allergen-induced late responses or histamine airway responsiveness, as measured either before or after inhaled allergen (Fig 2). The authors concluded that their findings "question the role of eosinophils in mediating the late asthmatic response and causing airway hyperresponsiveness".³⁷

A closer examination of the results presented in the paper, however, make these conclusions difficult to support. Treatment with the antibody clearly reduced blood and airway eosinophil numbers, thereby demonstrating the activity of the antibody and confirming a central role for IL-5 in the development of blood and airway eosinophilia after allergen inhalation in individuals with mild asthma. In contrast, the lack of effect of the treatment on the allergen-induced changes in airway function are not supported by the results described in the paper. There are 3 reasons for this:

1. The investigators did not demonstrate any obvious effect of allergen inhalation on histamine airway responsiveness on 2 of the 3 occasions when this was

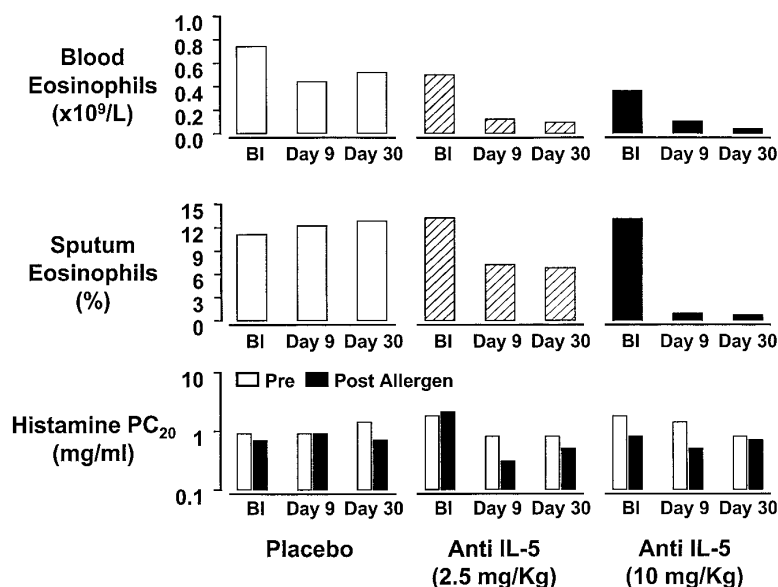


FIG 2. Effect of treatment with placebo or 2 doses of monoclonal antibody to IL-5 on blood and sputum eosinophils and histamine PC₂₀ before and 1 day after allergen inhalation. BI, Before inhalation. (Developed from data presented in: Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.)

measured at baseline in the treatment arms. The reported mean values for the provocative concentration of histamine causing a 20% fall in FEV₁ (PC₂₀) were 0.9 mg/mL before and 0.7 mg/mL the day after allergen in the placebo group, 1.8 mg/mL before and 2.1 mg/mL the day after allergen in the low-dose group, and 1.8 mg/mL before and 0.8 mg/mL the day after allergen in the high-dose group (Fig 2). In addition, the subjects did not develop any obvious allergen-induced AHR on 1 of the 2 occasions when this was measured during placebo treatment. The inability to demonstrate significant changes in allergen-induced AHR during the baseline period or during placebo treatment makes interpretation of the effects of active treatment on AHR impossible.

2. The main reason for measuring histamine airway responsiveness both before and 1 day after inhaled allergen is to compare the placebo and active treatments with respect to the allergen-induced *change* in airway responsiveness. This has been the standard approach in evaluating the effects of treatment, particularly in studies such as this, in which the baseline values varied both within and between each treatment group by more than the allergen-induced expected change in airway responsiveness (usually a mean doubling dose decrease in PC₂₀).³⁸⁻⁴¹ This statistical approach was not taken in the study in question; instead, the postallergen PC₂₀ values were compared between groups, the preallergen baseline being ignored. Indeed, if the usual approach had been taken, it would appear that the high-dose treatment was effective in preventing allergen-induced AHR on

day 29, when the change in histamine PC₂₀ during placebo treatment was from 1.4 to 0.7 mg/mL and the change during active treatment was from 0.8 to 0.7 mg/mL. Unfortunately, even if a formal statistical analysis were done in this way, the sample size would still be inadequate to have the study adequately powered for this outcome.⁴¹

3. The study was too underpowered to instill confidence with regard to the lack of effect of treatment on the physiologic outcomes. The sample size of 8 in each group was defended by the investigators with a quotation from a publication from our laboratory,⁴² in which the reproducibility of the late response was used to construct power curves, designed to ensure that studies such as this are adequately powered. All of the information used in these calculations was derived from randomized *crossover* studies, in which subjects were used as their own controls. When this design is used, a sample size of 8 subjects is adequate to have a power of >80% in detecting that an intervention has decreased the magnitude of the late response by 50%. However, these calculations cannot be used to estimate power in a study in which subjects are not being used as their own controls. Unfortunately, a measure of variance (SD or SE) of the late response was not provided in the paper by Leckie et al, so the actual power cannot be calculated. We have also published 2 analyses regarding the sample size requirements for demonstration of treatment effects on allergen-induced eosinophilia and increased airway responsiveness.^{41,43} Although both of these reports dealt with crossover designs, reexamination

of the data reveals that the sample size requirement for demonstrating a 50% attenuation of both of these outcomes with >80% power through use of a parallel-group design is 18 subjects in each treatment arm.

These issues illustrate the limitations of the study and of the conclusions that can be drawn from its results. Although we recognize (1) the complex interplay of various pathologic processes in asthma, (2) the possibility that airflow limitation, AHR, and airway inflammation represent different dimensions of the syndrome,⁴⁴ and (3) the limited ability of baseline airway eosinophilia to predict the bronchoconstrictor responses to inhaled allergen,⁴⁵ the study by Leckie et al³⁷ cannot be used either to support or to refute the concept of an important role for eosinophils in causing allergen-induced changes in airway function. Neither can the study argue against a role for cytokine-targeted therapy for asthma. These results have been interpreted by some commentators as definite proof that eosinophils are not directly involved in the pathogenesis of allergic asthma. This might be true, but given that the regulation of eosinophil production in the marrow, the circulation in peripheral blood, and the homing of these cells into the airway tissue (and their eventual spillover into the lumen) depend on a number of cytokines, chemokines, costimulatory molecules, and adhesion molecules, the appropriate study for reaching this conclusion has not yet been conducted. It is clear that a crossover study with an anti-IL-5 would be extremely difficult to undertake because of the very prolonged effect of the antibody on eosinophils; however, a parallel-group study would need approximately 20 subjects in each of the treatment groups before a definitive answer could be reached.

CONCLUSIONS

IL-5 is a pivotal mediator in the production and development of eosinophils. The weight of the evidence suggests that blocking IL-5 in sensitized mice prevents not only allergen-induced airway eosinophilia but also allergen-induced AHR. A study in allergic asthmatic subjects with a hmAb directed against IL-5 has confirmed that blocking IL-5 markedly reduces blood and airway eosinophils and that this effect is long-lasting. However, the study was not appropriately designed to evaluate the efficacy of the hmAb against allergen-induced airway responses.

The hmAb is a very exciting tool that will probably allow clarification of the role of airway eosinophils in asthma pathogenesis—when it is used with an appropriate study design.

REFERENCES

- Huber HL, Koessler KK. The pathology of bronchial asthma. *Arch Int Med* 1922;30:689-760.
- Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989;83:1013-26.
- Campbell HD, Tucker WQ, Hort Y, Martinson ME, Mayo G, Clutterbuck EJ, et al. Molecular cloning, nucleotide sequence, and expression of the gene encoding human eosinophil differentiation factor (interleukin 5). *Proc Natl Acad Sci U S A* 1987;84:6629-33.
- Sanderson CJ. Interleukin-5: an eosinophil growth and activation factor. *Dev Biol Stand* 1988;69:23-9.
- Clutterbuck EJ, Sanderson CJ. Human eosinophil hematopoiesis studied in vitro by means of murine eosinophil differentiation factor (IL-5): production of functionally active eosinophils from normal human bone marrow. *BMJ* 1988;71:646-51.
- Lopez AF, Sanderson CJ, Gamble JR, Campbell HD, Young IG, Vadas MA. Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J Exp Med* 1988;167:219-24.
- Wang JM, Rambaldi A, Biondi A, Chen ZG, Sanderson CJ, Mantovani A. Recombinant human interleukin 5 is a selective eosinophil chemoattractant. *Eur J Immunol* 1989;19:701-5.
- Sanderson CJ. The biological role of interleukin 5. *Int J Cell Cloning* 1990;8:147-53.
- Singh AD, Sanderson CJ. Anti-interleukin-5 strategies as a potential treatment for asthma. *Thorax* 1997;52:483-5.
- Roquet A, Dahlen B, Kumlin M, Ihre E, Aanstren G, Binks S, et al. Combined antagonist of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. *Am J Respir Crit Care Med* 1997;155:1856-63.
- O'Byrne PM, Dolovich J, Hargreave FE. Late asthmatic responses. *Am Rev Respir Dis* 1987;136:740-51.
- Gauvreau GM, Watson RM, O'Byrne PM. Kinetics of allergen-induced airway eosinophilic cytokine production and airway inflammation. *Am J Respir Crit Care Med* 1999;160:640-7.
- Gauvreau GM, Lee LM, Watson RM, Irani AM, Schwartz LB, O'Byrne PM. Increased numbers of both airway basophils and mast cells in sputum after allergen inhalation challenge in atopic asthmatics. *Am J Respir Crit Care Med* 2000;161:1473-8.
- Broide DH, Paine MM, Firestein GS. Eosinophils express interleukin 5 and granulocyte macrophage-colony-stimulating factor mRNA at sites of allergic inflammation. *J Clin Invest* 1992;90:1414-24.
- Sulakvelidze I, Inman MD, Rerecich TJ, O'Byrne PM. Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low dose allergen challenge in atopic asthmatics. *Eur Resp J* 1998;11:821-7.
- Hallden G, Hellman C, Gronneberg R, Lundahl J. Increased levels of IL-5 positive peripheral blood eosinophils and lymphocytes in mild asthmatics after allergen inhalation provocation. *Clin Exp Allergy* 1999;29:595-603.
- Sehmi R, Howie K, Sutherland DR, Shragge W, O'Byrne PM, Denburg JA. Increased levels of CD34 hemopoietic progenitor cells in atopic subjects. *Am J Respir Cell Mol Biol* 1996;15:645-54.
- Gibson PG, Girgis-Gabardo A, Dolovich J, Morris MM, Anderson M, Denburg J, et al. Blood eosinophils, basophils and progenitors during asthma exacerbations and resolution [abstract]. *Am Rev Respir Dis* 1989;139:482.
- Otsuka H, Dolovich J, Befus AD, Telizyn S, Bienenstock J, Denburg JA. Basophilic cell progenitors, nasal metachromatic cells and peripheral blood basophils in ragweed-allergic patients. *J Allergy Clin Immunol* 1986;78:365-71.
- Gibson PG, Manning PJ, O'Byrne PM, Girgis-Gabardo A, Dolovich J, Denburg JA, et al. Allergen-induced asthmatic responses. Relationship between increases in airway responsiveness and increases in circulating eosinophils, basophils, and their progenitors. *Am Rev Respir Dis* 1991;143:331-5.
- Wood LJ, Inman MD, Watson RM, Denburg JA, Foley J, O'Byrne PM. Bone marrow inflammatory progenitor cells after allergen inhalation in asthmatic subjects. *Am J Respir Crit Care Med* 1998;157:99-105.
- Sehmi R, Woods L, Watson RM, Foley R, Hamid Q, O'Byrne PM, et al. Allergen-induced increases in IL-5 a subunit expression on bone marrow derived CD34+ cells from asthmatic subjects: a novel marker of progenitor cell commitment towards eosinophil differentiation. *J Clin Invest* 1997;100:2466-75.
- Dent LA, Strath M, Mellor AL, Sanderson CJ. Eosinophilia in mice expressing interleukin 5. *J Exp Med* 1990;172:1425-31.
- Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med* 1997;185:2143-56.
- Foster PS, Hogan SP, Ramsay AJ, Matthaei 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.
26. Mathur M, Herrmann K, Li X, Qin Y, Weinstock J, Elliott D, et al. TRFK-5 reverses established airway eosinophilia but not established hyperresponsiveness in a murine model of chronic asthma. *Am J Respir Crit Care Med* 1999;159:580-7.
27. 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;161:1501-9.
28. Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Sheppard D, et al. Requirements for allergen-induced airway hyperreactivity in T and B cell-deficient mice. *Mol Med* 1998;4:344-55.
29. Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. *Science* 1998;282:2258-61.
30. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Rennick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. *Science* 1998;282:2261-3.
31. Tournoy KG, Kips JC, Schou C, Pauwels RA. Airway eosinophilia is not a requirement for allergen-induced airway hyperresponsiveness. *Clin Exp Allergy* 2000;30:79-85.
32. Shardonofsky FR, Venzor J III, Barrios R, Leong KP, Huston DP. Therapeutic efficacy of an anti-IL-5 monoclonal antibody delivered into the respiratory tract in a murine model of asthma. *J Allergy Clin Immunol* 1999;104:215-21.
33. 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;75:284-8.
34. Karras JG, McGraw K, McKay RA, Cooper SR, Lerner D, Lu T, et al. Inhibition of antigen-induced eosinophilia and late phase airway hyperresponsiveness by an IL-5 antisense oligonucleotide in mouse models of asthma. *J Immunol* 2000;164:5409-15.
35. 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;161:1501-9.
36. Tournoy KG, Kips JC, Pauwels RA. The allergen-induced airway hyperresponsiveness in a human-mouse chimera model of asthma is T cell and IL-4 and IL-5 dependent. *J Immunol* 2001;166:6982-91.
37. Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
38. Kirby JG, Hargreave FE, Cockcroft DW, O'Byrne PM. Effect of indomethacin on allergen-induced asthmatic responses. *J Appl Physiol* 1989;66:578-83.
39. Cockcroft DW, Murdock KY, Gore BP, O'Byrne PM, Manning P. Theophylline does not inhibit allergen-induced increase in airway responsiveness to methacholine. *J Allergy Clin Immunol* 1989;83:913-20.
40. Gauvreau GM, Doctor J, Watson RM, Jordana M, O'Byrne PM. Effects of inhaled budesonide on allergen-induced airway responses and airway inflammation. *Am J Respir Crit Care Med* 1996;154:1267-71.
41. Inman MD, Hamilton AL, Kerstjens HA, Watson RM, O'Byrne PM. The utility of methacholine airway responsiveness measurements in evaluating anti-asthma drugs. *J Allergy Clin Immunol* 1998;101:342-8.
42. Inman MD, Watson R, Cockcroft DW, Wong BJ, Hargreave FE, O'Byrne PM. Reproducibility of allergen-induced early and late asthmatic responses. *J Allergy Clin Immunol* 1995;95:1191-5.
43. Gauvreau GM, Watson RM, Rerecich TJ, Baswick E, Inman MD, O'Byrne PM. Repeatability of allergen-induced airway inflammation. *J Allergy Clin Immunol* 1999;104:66-71.
44. Rosi E, Ronchi MC, Grazzini M, Duranti R, Scano G. Sputum analysis, bronchial hyperresponsiveness, and airway function in asthma: results of a factor analysis. *J Allergy Clin Immunol* 1999;103(2 Pt 1):232-7.
45. Avila PC, Segal MR, Wong HH, Boushey HA, Fahy JV. Predictors of late asthmatic response. Logistic regression and classification tree analyses. *Am J Respir Crit Care Med* 2000;161:2092-5.