

Early reports

Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response

Margaret J Leckie, Anneke ten Brinke, Jamey Khan, Zuzana Diamant, Brian J O'Connor, Christine M Walls, Ashwini K Mathur, Hugh C Cowley, K Fan Chung, Ratko Djukanovic, Trevor T Hansel, Stephen T Holgate, Peter J Sterk, Peter J Barnes

Summary

Background Interleukin-5 (IL-5) is essential for the formation of eosinophils, which are thought to have a major role in the pathogenesis of asthma and other allergic diseases. We aimed to assess the effects of monoclonal antibody to IL-5 on blood and sputum eosinophils, airway hyper-responsiveness, and the late asthmatic reaction to inhaled allergen in patients with mild asthma.

Methods We did a double-blind randomised placebo-controlled trial, in which a single intravenous infusion of humanised (IgG-κ) monoclonal antibody to IL-5 (SB-240563) was given at doses of 2.5 mg/kg (n=8) or 10.0 mg/kg (n=8). The effects of treatment on responses to inhaled allergen challenge, sputum eosinophils, and airway hyper-responsiveness to histamine were measured at weeks 1 and 4 with monitoring of blood eosinophil counts for up to 16 weeks.

Findings Monoclonal antibody against IL-5 lowered the mean blood eosinophil count at day 29 from $0.25 \times 10^9/L$ (95% CI 0.16–0.34) in the placebo group to $0.04 \times 10^9/L$ (0.00–0.07) in the 10 mg/kg group ($p < 0.0001$), and prevented the blood eosinophilia that follows allergen challenge. After inhaled allergen challenge, 9 days after treatment, the percentage sputum eosinophils were 12.2% in the placebo group and lowered to 0.9% (–1.2 to 3.0; $p = 0.0076$) in the 10 mg/kg group, and this effect persisted at day 30 after the dose. There was no significant effect of monoclonal antibody to IL-5 on the late asthmatic response or on airway hyper-responsiveness to histamine.

Interpretation A single dose of monoclonal antibody to IL-5 decreased blood eosinophils for up to 16 weeks and sputum eosinophils at 4 weeks, which has considerable therapeutic potential for asthma and allergy. However, our findings question the role of eosinophils in mediating the late asthmatic response and causing airway hyper-responsiveness.

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National Heart and Lung Institute (NHLI), Imperial College, London SW3 6LY, UK (M J Leckie, MRCP, J Khan BSc, B J O'Connor MRCPI, T T Hansel FRCP, Prof K F Chung FRCP, Prof P J Barnes FRCP); **Department of Pulmonology, Leiden University Medical Centre, Leiden, Netherlands** (A ten Brinke MD, Z Diamant MD, Prof P J Sterk MD); **Clinical Pharmacology, SmithKline Beecham, Harlow, UK** (C M Walls MSc, A K Mathur PhD, H C Cowley FRCA); **Respiratory Cell and Molecular Biology Research Division, University of Southampton, UK** (R Djukanovic MD, Prof S T Holgate FRCP)

Correspondence to: Prof Peter J Barnes (e-mail: p.j.barnes@ic.ac.uk)

Introduction

Asthma is a chronic inflammatory disease of the airways in which eosinophils are prominent in sputum, bronchoalveolar lavage, and mucosal biopsy samples.¹ These tissue eosinophils are terminally differentiated myeloid leucocytes with characteristic secondary granules² that have the capacity to cause airway hyper-responsiveness in bronchial smooth-muscle.³ Furthermore, eosinophils could mediate some of the structural changes comprising airways remodelling, including epithelial shedding, sub-basement membrane collagen deposition, and smooth-muscle hypertrophy.⁴

Within the bone marrow, interleukin-5 (IL-5) is the major haematopoietin responsible for terminal differentiation of human eosinophils.⁵ This cytokine could be involved in eosinophilic inflammation in asthma⁶ since there are raised concentrations of IL-5 in serum⁷ and bronchial biopsy samples.⁸ In addition, raised serum IL-5 and blood eosinophils are associated with the fall in forced expiratory volume in 1 s (FEV₁) that constitutes the late asthmatic reaction.⁹

Direct evidence that IL-5 might be harmful in asthma is that inhalation of IL-5 by patients with asthma causes airway hyper-responsiveness and sputum eosinophilia.¹⁰ Monoclonal antibodies to IL-5 administered to animals with allergic asthma cause long-term inhibition of pulmonary eosinophilia and airway hyper-responsiveness.^{11,12} Furthermore, mice in which the IL-5 gene has been deleted failed to develop pulmonary eosinophilia and airway hyper-responsiveness after allergen challenge.¹³

We aimed to describe the effect of a single intravenous infusion of humanised monoclonal antibody against IL-5 on blood and sputum eosinophils, and the responses to inhaled allergen in patients with atopic asthma. In particular, our study was designed to assess the tolerability and activity of a potent and specific anti-eosinophil treatment.

Methods

Patients

We recruited 24 non-smoking men (mean age 27, range 18–45 years) with mild allergic asthma (as defined by the American Thoracic Society)¹⁴ and a history of episodic wheeze and shortness of breath. The patients were atopic, as defined by positive skin tests in response to common airborne allergens (*Dermatophagoides pteronyssinus*, mixed grass pollen, and cat hair) and were maintained on short-acting inhaled β_2 -agonist treatment as required (table 1). Patients had neither worsening asthma nor a respiratory infection in the preceding 6 weeks. FEV₁ at baseline was at least 70% of the predicted value and there was a documented airway hyper-responsiveness to histamine, with a provocation concentration causing a 20% reduction in FEV₁ (PC₂₀) < 8 mg/mL. Patients had

	Placebo (n=8)	2.5 mg/kg dose (n=8)	10 mg/kg dose (n=8)
Age (years)	25.6 (4.1)	30.0 (8)	28.0 (4.3)
FEV ₁ (% predicted)	93 (9.6)	90.3 (10.4)	82.0 (7)
Maximum late asthmatic reaction (FEV ₁ % reduction from baseline)	39.9 (14.4)	32.1 (11.1)	33.6 (11.9)
PC ₂₀ (mg/L; preallergen challenge)	0.9 (0.4)	1.8 (1.4)	1.8 (2.1)
Blood eosinophils ($\times 10^9/L$; preallergen challenge)	0.38 (0.15)	0.20 (0)	0.3 (0.12)
Sputum eosinophils (% of leucocytes)	11.1 (11.5)	13.2 (11.1)	13.1 (10)

Values shown as mean (SD). FEV₁=forced expiratory volume in 1 s.

PC₂₀=provocative concentration of histamine.

Table 1: Baseline characteristics of the 24 patients

documented early and late asthmatic responses (defined as a $\geq 15\%$ reduction in FEV₁ on at least three occasions between 4 and 10 h after allergen) to inhaled incremental allergen challenge between 3 and 6 weeks before the study treatment was given. We obtained approval from relevant national regulatory agencies, local ethics committees, and written informed patient consent.

Study design

This study was a double-blind, randomised, placebo-controlled, single dose, parallel group study in three centres. At screening (28 days before the dose), a full medical history, examination, and investigations were done to ensure eligibility for the study. We examined stool specimens for absence of parasites. 14 days before the dose, patients had a histamine challenge followed 2 h later by an inhaled allergen challenge, with a bolus dose equal to the sum of the incremental doses that produced the screening late asthmatic reaction. 22 h after allergen inhalation, a further histamine challenge was done and sputum was induced 1 h later. On day 1, patients were randomly assigned intravenous infusion of humanised monoclonal antibody to IL-5 (SB 240563) or placebo. Allocation to active treatment or placebo was in a 2:1 ratio for the 2.5 mg/kg and 10 mg/kg groups (n=12 for each group, eight receiving monoclonal antibody to IL-5 and four receiving placebo). A 30 min intravenous infusion of either study treatment or placebo was done, with continuous electrocardiographic monitoring, and measurement of blood pressure and lung function at regular intervals. 24 h after the infusion, patients were discharged, but they returned on 2 successive days for blood analysis and lung function testing. On day 8 and 29 after the dose, all patients had a histamine challenge followed 2 h later by an inhaled allergen challenge with another histamine challenge, and sputum induction the next day. Further visits were made at intervals up to 16 weeks after the dose for assessment of airway hyper-responsiveness and blood eosinophil counts.

All the 24 patients assigned placebo in both dose groups completed all of the visits up to day 30. One patient in the 2.5 mg/kg group withdrew for personal reasons at week 12 after the dose (figure 1).

Procedures

We analysed blood eosinophil counts with automated haematology analysers at each of the participating centres. For bolus allergen challenge, a single volume of relevant allergen (ALK, Reading, UK) was diluted with saline to make up a single dose, which was equal to the sum of the incremental doses inhaled by the patient to cause the early phase during the screening allergen challenge.¹⁵ We measured airway hyper-responsiveness using inhalation of doubling concentrations of histamine according to standard challenge protocols.¹⁶

Before sputum induction, all patients inhaled 200 μ g of salbutamol via a metered dose inhaler. The patients inhaled hypertonic saline to induce sputum, and selected sputum was analysed.¹⁷ Cytospins of sputum cells were prepared, fixed with methanol, and stained by May-Grunwald-Giemsa.

Statistical analysis

We analysed baseline variables by analysis of variance. For the primary variable of late asthmatic response, the maximum percentage change in FEV₁ from the dose at 4 to 10 h after allergen challenge was calculated for days -14, 8, and 29. An analysis of covariance (ANCOVA) model accounting for baseline value (-14 days), group (placebo, 2.5 mg/kg, and 10 mg/kg), time (day 8 and day 29), and interaction between group and time was used. Least squares means for day 8 and day 29 and the mean differences between placebo and the two treated groups on day 8 and day 29 are reported along with the 95% CIs. We did similar analyses separately for blood eosinophils, sputum eosinophils, provocative concentration of histamine preallergen challenge and postallergen challenge, but eosinophils were analysed on the square root scale whereas histamine was analysed on the log scale. For these transformed variables, the least squares means, mean differences, and the associated CIs are back-transformed to the original scale, and these are reported (table 2).

Results

In the 24 patients who participated in the study (table 1), treatment with monoclonal antibody treatment to IL-5 at baseline (day -14) caused no clinically relevant adverse events. Antibodies to IL-5 did not develop during the 16 week study period.

At baseline (day -14) for FEV₁ (% predicted) the difference between the placebo and the 2.5 mg/kg groups was not significant, but there was a small significant difference between the placebo and the 10 mg/kg groups

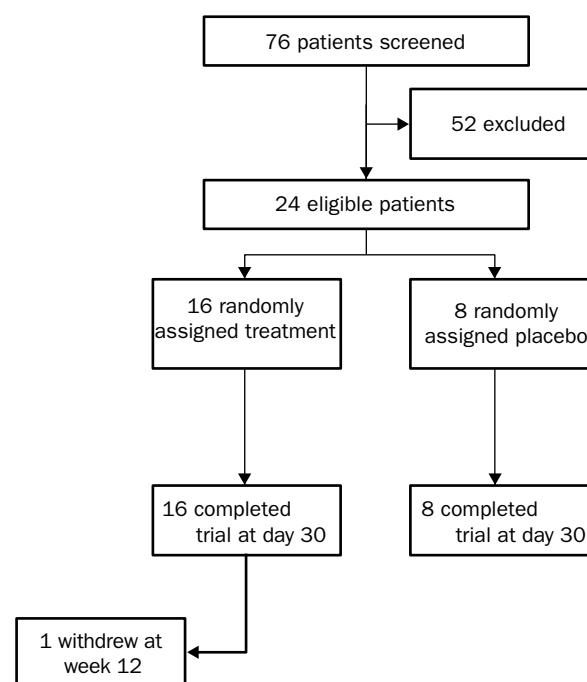


Figure 1: Trial profile

Placebo		Monoclonal antibody to IL-5		Difference		p-value by ANCOVA	
		2.5 mg/kg	10 mg/kg	Placebo vs 2.5 mg/kg	Placebo vs 10 mg/kg	Placebo vs 2.5 mg/kg	Placebo vs 10 mg/kg
Blood eosinophils (×10⁹/L) preallergen							
Day -14 (baseline)	0.38	0.20	0.30	0.18 (0.01 to 0.36)	0.08 (-0.09 to 0.26)	0.0292	0.4960
Day 8	0.26	0.24	0.08	0.01 (-0.16 to 0.19)	0.17 (0.04-0.30)	1.0000	0.0054
Day 29	0.25	0.23	0.04	0.02 (-0.14 to 0.18)	0.21 (0.10-0.33)	1.0000	<0.0001
Blood eosinophils (×10⁹/L) postallergen							
Day -13 (baseline)	0.74	0.50	0.36	0.23 (-0.11 to 0.58)	0.38 (0.07-0.69)	0.2136	0.0144
Day 9	0.44	0.12	0.10	0.32 (0.11-0.53)	0.34 (0.13-0.55)	0.0012	0.0006
Day 30	0.52	0.09	0.04	0.43 (0.22-0.65)	0.49 (0.28-0.70)	0.0002	<0.0001
Sputum eosinophils (%)							
Day -13 (baseline)	11.1	13.2	13.1	-2.1 (-16.3 to 12.2)	-2.0 (-16.2 to 12.3)	1.0000	1.0000
Day 9	12.2	7.2	0.9	5.0 (-5.9 to 16.0)	11.3 (2.6-20.1)	0.6108	0.0076
Day 30	12.8	6.8	0.7	5.9 (-5.0 to 16.8)	12.1 (3.1-21.0)	0.4454	0.0050
Histamine PC₂₀ (mg/ml) preallergen							
Day -14 (baseline)	0.9	1.8	1.8	-0.9 (-2.8 to 1.0)	-0.9 (-2.8 to 1.0)	0.5292	0.5324
Day 8	0.9	0.8	1.4	0.1 (-1.0 to 1.2)	-0.5 (-1.9 to 0.9)	1.0000	0.9142
Day 29	1.4	0.8	0.8	0.6 (-0.8 to 2.0)	0.6 (-0.7 to 2.0)	0.6744	0.6062
Histamine PC₂₀ (mg/ml) postallergen							
Day -13 (baseline)	0.7	2.1	0.8	-1.4 (-3.2 to 0.5)	-0.1 (-1.9 to 1.7)	0.1716	1.0000
Day 9	0.9	0.3	0.5	0.6 (-0.2 to 1.3)	0.4 (-0.5 to 1.2)	0.7008	0.6022
Day 30	0.7	0.5	0.7	0.2 (-0.4 to 0.9)	0.1 (-0.7 to 0.7)	0.9248	1.0000
Late asthmatic reaction (maximum % fall in FEV₁)							
Day -14 (baseline)	42.9	32.3	33.6	10.7 (-3.4 to 24.7)	9.3 (-5.1 to 23.8)	0.1610	0.2654
Day 8	34.5	33.9	31.7	0.6 (-10.0 to 11.2)	2.8 (-8.2 to 13.7)	1.0000	1.0000
Day 29	32.5	36.0	32.8	-3.5 (-10.4 to 3.5)	-0.3 (-7.1 to 6.5)	0.5050	1.0000

p values and 95% CI for mean differences are Bonferroni corrected to account for two pairwise comparisons to the placebo group at each day. PC₂₀=provocative concentration of histamine. Baseline differences were accounted for.

Table 2: Effects of monoclonal antibody to IL-5 after allergen challenge

($p=0.0494$). There was a significant difference between the placebo group and the 2.5 mg/kg group for preallergen-challenge blood eosinophil counts ($p=0.0292$), but this difference was not significant between the placebo and the 10 mg/kg group. At day -13 for postallergen-challenge blood eosinophil counts, the difference between the placebo group and the 2.5 mg/kg was not significant, but there was a significant difference between the placebo group and the 10 mg/kg group ($p=0.0144$).

Numbers of venous blood eosinophils were substantially lowered in all patients given monoclonal antibody to IL-5 (figure 2, table 2). Blood eosinophils were higher in the placebo group compared with the 10 mg/kg group at day 9 ($p=0.0006$) and day 30 ($p<0.0001$) after treatment. As shown in figure 2, this suppression of eosinophil numbers was maintained up to 16 weeks after the dose. In the 2.5 mg/kg group there was a similar, although less profound fall in blood eosinophil numbers, which was maintained for 30 days after the dose.

There was also a dose-dependent decrease in sputum eosinophils, which lasted for 30 days after the dose (figure 3). At day 9, sputum eosinophils were higher in the placebo group, than in the 10 mg/kg group ($p=0.0076$), which was maintained at day 30 ($p=0.0050$).

All patients had a PC₂₀ to histamine of less than 8 mg/mL before allergen challenge at baseline. Monoclonal antibody to IL-5 at doses of 2.5 mg/kg and 10 mg/kg caused no significant changes in airway responsiveness to histamine before and after allergen challenges on days 8 and 29 (figure 3, table 2).

At baseline all patients had a documented early and late response to allergen challenge. However, patients in the placebo group had a more pronounced maximum late asthmatic reaction than those in the 10 mg/kg dose group at baseline, and this trend was maintained at day 8 after the dose (table 2). The preceding challenge with inhaled histamine did not seem to change the magnitude of the

early or the late asthmatic reaction since a previous study using identical allergen challenge methodology, but without preceding histamine challenge showed similar magnitude of both.¹⁸ Treatment with monoclonal antibody against IL-5 caused no attenuation of the early or the maximum late asthmatic reaction in either dose group (table 2).

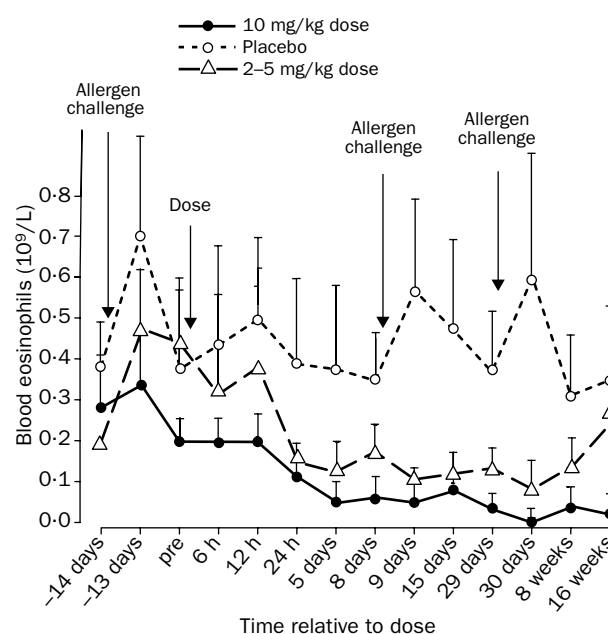


Figure 2: Effect of monoclonal antibody to IL-5 on blood eosinophil number

There were eight patients per group up to day 30, after which sample sizes were smaller. Raw data is expressed as arithmetic mean with 95% CI.

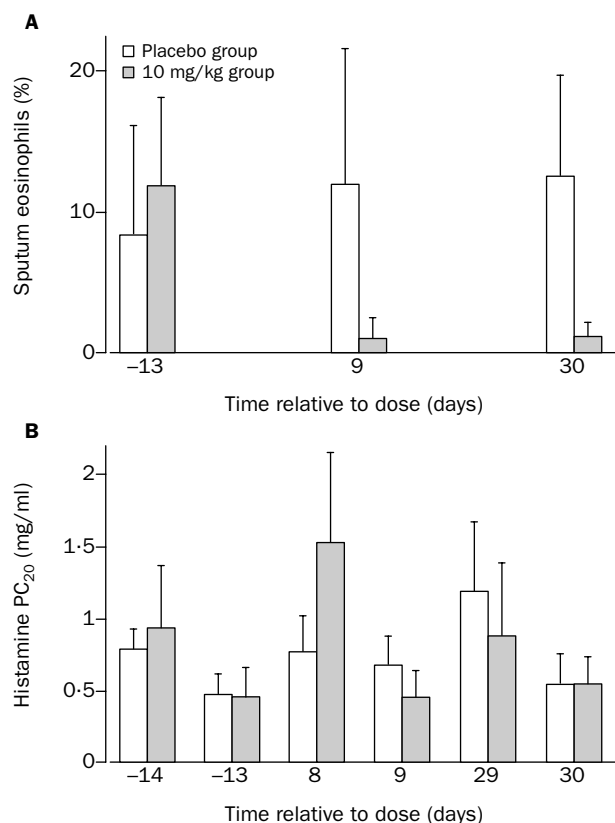


Figure 3: Effect of monoclonal antibody to IL-5 on sputum eosinophils and histamine PC₂₀

A: mean % of total leucocytes. B: Histamine PC₂₀ was done before inhaled allergen challenge on day -14, 8, and 29, and repeated 24 days later. Raw data is expressed as arithmetic mean with 95% CI for sputum eosinophils and geometric mean with geometric SE for histamine PC₂₀.

Discussion

A single intravenous infusion of monoclonal antibody against IL-5 given to patients with allergic asthma caused pronounced long-term suppression of circulating eosinophils, and greatly lowered the degree of sputum eosinophilia after allergen challenge. However, despite these clear effects, the treatment did not protect against the allergen-induced late asthmatic response and did not have effects on baseline or postallergen airway hyper-responsiveness. This finding suggests that eosinophils might not be a prerequisite for the late asthmatic response and airway hyper-responsiveness in relation to inhaled allergen challenge, and has relevance to the pathogenesis and treatment of asthma.

The striking decrease in blood and sputum eosinophil numbers after monoclonal antibody to IL-5 has potential for the treatment of asthma, allergic rhinitis, and atopic dermatitis. The long duration of action after a single dose is particularly beneficial in asthma when compliance with current treatment is often poor.¹⁹ Monoclonal antibody versus IL-5 might be especially effective for the treatment of eosinophilic childhood asthma, and when used early in the natural history could inhibit the structural changes of airways remodelling. Humanised monoclonal antibody to IL-5 was well tolerated, but treatment of this type could theoretically cause allergic reactions and compromise host defence against parasitic infestations.

In this study, the reproducibility of the late asthmatic reaction is such that the statistical power is sufficient to exclude any major effect of monoclonal antibody against IL-5 on the reaction.²⁰ The generation of the late

asthmatic reaction by the concerted action of T cells, mast cells, eosinophils, and other cells could explain the lack of inhibition of the reaction. Redundancy between different cells in generating the late asthmatic reaction is shown by such diverse agents as corticosteroids,²¹ ciclosporin A,²² systemic anti-IgE,²³ and leucotriene antagonists²⁴ all being able to inhibit the reaction. Involvement of various cell types and mediators, and the activity of low numbers of residual eosinophils in the airways could be the basis for eosinophil-directed treatments not readily suppressing the late asthmatic reaction.

Animal studies provide conflicting data on the association of eosinophilic inflammation with airway hyper-responsiveness.^{25,26} A dissociation between eosinophil numbers and airway hyper-responsiveness has been shown in the guinea-pig, in which low doses of monoclonal antibody against IL-5 can decrease eosinophils but not airway hyper-responsiveness.²⁷ Treatment with monoclonal antibody to IL-5 has not been shown to reverse established airway hyper-responsiveness in mice.²⁸ Furthermore, in Brown-Norway rats ciclosporin A completely suppresses the eosinophilic inflammation induced by allergen but has no effect on airway hyper-responsiveness, whereas a corticosteroid effectively suppresses bronchial eosinophils and hyper-responsiveness.²⁹ In addition, mice deficient of IL-4 and IL-5 had persistent hyper-responsiveness despite a lack of inflammatory infiltrate and the response in these mice was abolished on injection with anti-CD4.³⁰

Clinical studies in human allergic asthma have also shown dissociation between airway inflammation, airway hyper-responsiveness, and the late asthmatic reaction.^{31,32} It has been shown recently that in eight asthmatics with an allergy to cats, although intradermal injection of peptide results in a late asthmatic reaction, bronchoalveolar lavage and bronchial biopsy samples did not have increased numbers of Th-2 lymphocytes or eosinophils, suggesting a dissociation between cellular infiltration and airway narrowing.³³ By contrast with monoclonal antibody to IL-5, inhaled steroids have considerable effects on eosinophils, the late asthmatic reaction, and airway hyper-responsiveness in patients with asthma with similar characteristics to those involved in our study.²¹ This finding has implications for development of new treatments for asthma, since if drugs are used to counter eosinophilic inflammation, additional treatment might be required to counter airway hyper-responsiveness.

A single intravenous infusion of monoclonal antibody to IL-5 is well tolerated, causes a long-term decrease in both peripheral blood and sputum eosinophils, and has treatment potential for diseases associated with eosinophilia, although a preliminary report of a differing monoclonal antibody against IL-5 in severe asthma did not show convincing efficacy.³⁴ Monoclonal antibody versus IL-5 and other specific anti-eosinophil treatments could have limited effects on the late asthmatic reaction and airway hyper-responsiveness because of the involvement of a number of other cell types in these responses. A large-scale clinical study on the efficacy of multiple doses monoclonal antibody against IL-5 will provide further insight into the role of eosinophils in the clinical pathogenesis of allergy and asthma.

Contributors

J Khan undertook sputum processing and analysis, A Mathur contributed to data processing and statistical analysis. All other investigators were involved in protocol design and clinical aspects of carrying out this study. Peter Barnes was principal investigator. All investigators contributed to writing the manuscript.

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