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Airway inflammation in asthma : from concept to the clinic

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Chapter
2

Evidence for systemic rather than pulmonary effects of interleukin-5 administration in asthma

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Abstract

Background Interleukin-5 (IL-5) plays an important role in mobilisation of eosinophils from the bone marrow and in their subsequent terminal differentiation. We investigated whether IL-5 could induce pulmonary eosinophilia and bronchial hyperresponsiveness (BHR) independently of these effects by examining the effects of inhaled and intravenous administration of IL-5.

Methods Nine mild asthmatics received in random order inhaled (15µg) or intravenous (2µg) IL-5 or placebo in a double blind, crossover study. Blood samples were taken prior to and at 1/2, 1, 2, 3, 4, 5, 24 and 72 hours following IL-5 or placebo, and PC₂₀ (methacholine) and eosinophil counts in induced sputum were determined.

Results Serum IL-5 was markedly increased 30 min after intravenous IL-5 (p=0.002), and sputum IL-5 increased 4 and 24 hours after inhaled IL-5 (p<0.05). Serum eotaxin was elevated 24 hours after intravenous but not inhaled IL-5 or placebo. Blood eosinophils were markedly reduced from 0.5 – 2 hours following intravenous IL-5 (p<0.05), followed by an increase at 3, 4, 5 and 72 hours (p<0.05). Sputum eosinophils rose significantly in all three groups at 24 hours but there were no between-group differences. Bronchial responsiveness (PC₂₀) was not affected by IL-5.

Conclusion The effects of IL-5 appear to be mainly in the circulation, inducing peripheral mobilisation of eosinophils to the circulation, without effect on eosinophil mobilisation in the lungs and on bronchial responsiveness.

Introduction

Chronic asthma is characterised by an inflammation of the bronchi with the presence of eosinophils and CD4+ T-helper cells (1,2). T-helper type 2 (Th₂) cells are the predominant source of the cytokine IL-5 and are elevated in the asthmatic airways (2). IL-5 plays an important role in the mobilisation of eosinophils from the bone marrow, and in the terminal differentiation and maintenance of mature eosinophils (3). IL-5 levels have been measured in the circulation of asthmatics (4), and rises during asthma exacerbations (1). Additionally, increased IL-5 mRNA expression is seen in bronchial mucosa and bronchoalveolar lavage CD4+ T cells in asthma (2,5), and IL-5 protein levels increase following allergen challenge (6). A positive correlation between asthma severity and IL-5 mRNA levels in bronchial biopsies has also been demonstrated (7).

In guinea pig and rodent models, airway administration of IL-5 induces an increase in airway eosinophils without demonstrable change in bronchial responsiveness (8,9). Airway and blood eosinophilia has also been demonstrated in IL-5 transgenic mice, but without bronchial hyperresponsiveness (10-12). IL-5 administration to the airways of asthmatic subjects, however, has been associated with both peripheral blood eosinophilia, airway eosinophilia and bronchial hyperresponsiveness (13;14).

These conflicting observations suggest a complex co-operative role for IL-5 in the accumulation of circulating and airway eosinophils and their possible subsequent contribution to bronchial hyperresponsiveness. Since IL-5 also appears to have important effects on the bone marrow (9,15,16), we questioned whether the route of IL-5 production was crucial to its effects on the airways. We hypothesised that IL-5 confined entirely to the lungs and airways would have little systemic effect on eosinophil mobilisation, while IL-5 administered to the circulation might possess this primary effect. Further, we sought to establish whether pulmonary or systemic IL-5 could induce specific pulmonary effects consistent with asthma. We therefore investigated the effects of IL-5 administered intravenously or by inhalation to patients with mild asthma on eosinophil counts in blood and in sputum, and on airway hyperresponsiveness.

Methods

Subjects

Nine non-smoking, atopic patients with mild persistent asthma (5 women, age range 25-41 years) participated in this study (Table 1). All subjects had a history of episodic chest tightness, and wheezing and symptoms were controlled by on-demand usage of short-acting inhaled β_2 -agonists alone. All subjects were free of respiratory tract infection symptoms for at least 2 weeks prior to study commencement. Atopy was determined by positive skin-prick responses (>3 mm) to extracts of 6 common aeroallergens (Vivodiagnost, ALK, Benelux). Baseline forced expiratory volume in 1 second (FEV₁) was $>70\%$ of predicted (17) and bronchial hyperresponsiveness (PC₂₀ < 8 mg/ml

Table 1. Subject characteristics of mild asthma patients

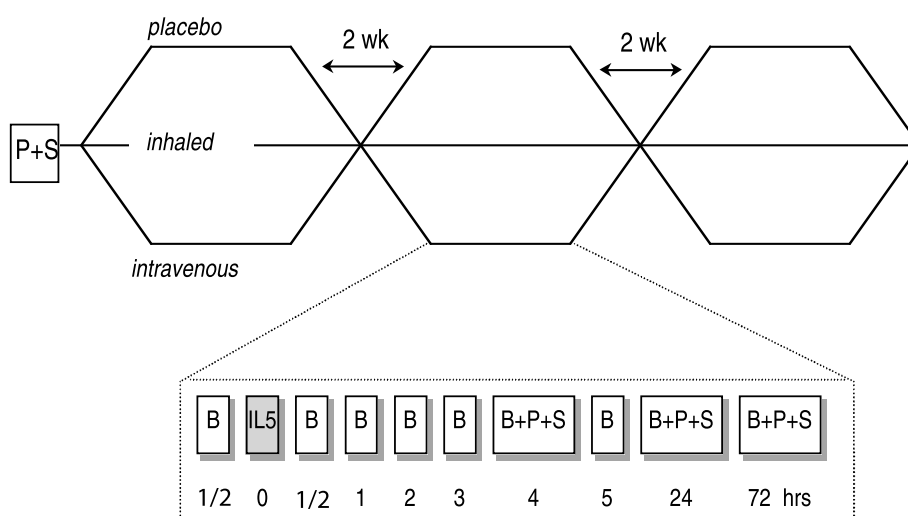
Subject	Sex	Age (years)	FEV ₁ (% pred)	PC ₂₀ (mg/ml)	Sputum Eosinophils (%)
1	M	35	94	0.15	2.3
2	F	26	85.6	0.19	0.5
3	F	26	79	0.37	6.3
4	F	27	95	0.42	1.1
5	F	36	97	1.44	1.0
6	F	25	117	2.79	0.0
7	M	29	106	3.73	5.9
8	M	28	97	6.68	0.5
9	M	41	85	8.00	0.2
		30.3 ± 5.6*	95.1 ± 3.8*	1.15 ± 2.21#	1.00 (0-6.3)†

* mean-SEM; # geometric mean-SD in doubling doses; † median (range).

methacholine), were determined by standardised technique (18). The protocol was approved by the Ethics Committee of the Royal Brompton Hospital and all participants gave written informed consent.

IL-5 administration

Recombinant human IL-5 (Genzyme, Boston, MA) was reconstituted in 0.9% NaCl (2ml) and administered at a dose of 2 µg intravenously via a slow infusion in a forearm vein



IL5 = administration of inhaled (15 µg), intravenous (2 µg) IL-5 or placebo

B = blood sample

P = PC₂₀ methacholine

S = sputum induction

Figure 1. Study design. Subjects were treated in random order with inhaled IL-5, intravenous IL-5 or placebo, in a cross-over design at 2 week intervals. Specimen sampling was performed at time-points noted.

over a period of 5 mins. This intravenous dose was calculated according to the levels of circulating IL-5 measured in circulating blood of patients with asthma during an exacerbation (1). IL-5 (15µg) was inhaled over 5 minutes via a nebulizer (MECIC-AID, Pagham, Sussex, UK) from a mouthpiece using a one-way exhaust valve (LC Plus, PARI, Surrey, UK). To ensure complete inhalation of the IL-5, the chamber was then refilled with 2ml 0.9% NaCl and the nebulisation repeated.

Lung function and methacholine challenge

Baseline FEV₁ was recorded from the best of three attempts using a dry wedge spirometer (Vitalograph, Buckingham, UK) (17). Spirometry was performed prior to and hourly after the administration of IL-5 or placebo until the start of methacholine challenge at 4h. Methacholine challenges were performed according to a standardised methodology (18). After a nebulized saline challenge, doubling doses of methacholine (0.06-32 mg/ml) were administered via a dosimeter (Mefar, Bovezzo, Italy) with an output of 100µl. A total of five inhalations of each concentration was administered (inhalation time one sec and breath-holding time six sec). FEV₁ was measured 2 and 3 min after each dose. The test was discontinued if FEV₁ as compared with the control inhalation (0.9% NaCl) decreased by 20% or more. The PC₂₀ was calculated by log-linear interpolation of the last two data-points.

Peripheral blood eosinophil counts

Peripheral blood eosinophils were identified by the combination of peroxidase staining and side scatter and enumerated using the automated Advia 120 Hematology System (Bayer, Newbury, Berks, UK).

Sputum induction

All subjects received 200µg salbutamol following the methacholine challenge. After recovery, sputum was induced according to a previously-described method (19). Hypertonic saline aerosols (NaCl 3.5%) were nebulised at room temperature via an ultrasonic nebuliser (DeVilbiss Ultraneb 2000) at maximum output. Subjects inhaled the aerosols during 3 x 5-minute intervals. Between each interval, or as soon as the subjects started coughing, they were instructed to wash their mouth and blow their nose in order to minimise salivary contamination. The induced sputum was collected into a 50-ml tube, kept at 4°C and processed within two hours.

Sputum processing

The whole sputum sample was diluted with 2 ml Hank's balanced salt solution (HBSS) containing 0.25% dithiotreitol (DTT; Sigma Chemicals, Poole, UK). The sample was gently mixed at room temperature, the volume determined and the sample further diluted with HBSS to 10 ml. The sample was centrifuged (350g, 10 min), supernatant was removed and the cell pellet resuspended in 1 ml HBSS. Total cell counts were determined on a haemocytometer slide. Cytospin slides were prepared (600 rpm, 6 min; Shandon, Runcorn, UK) and stained with May-Grunwald-Giemsa. Differential cell counts were performed by a blinded observer with 300 non-squamous cells counted on each of

two slides. To correct for the variable salivary contamination, differential cell counts were expressed as a percentage of 300 nucleated cells, excluding squamous epithelial cells. An adequate sample was defined if there was < 80% squamous cell contamination. Supernatants were stored at -80°C .

IL-5 and eotaxin assays

Serum IL-5 and eotaxin and sputum IL-5 levels were measured by ELISA according to the manufacturer's instructions (Pharmingen, Cambridge, UK). Briefly, purified rat anti-human monoclonal antibodies were incubated at $2\ \mu\text{g}/\text{ml}$ in coating solution (0.1M NaHCO_3 (BDH) pH 8.2 at 4°C) overnight. Plates were washed then blocked with 10% FCS for 2h at room temperature. Sample supernatants were added to each plate in duplicate. Cytokine standards (R&D Systems, Abingdon, Oxon, UK) were diluted in 10% (v/v) FCS/PBS/Tween and added in duplicate. Plates were then incubated at 4°C overnight. Biotinylated rat anti-human antibody (R&D) was added and incubated at room temperature for 45 mins then washed before the addition of avidin-peroxidase. Plates were incubated at room temperature for 30mins before washing with PBS/Tween and (ABTS - Sigma) substrate. Plates developed for approximately 10 minutes before measurement on a plate reader (Anthos Labtec, Austria) at 405nm. The detection limit of this assay was $35\ \text{pg}/\text{ml}$.

Data analysis

PC_{20} was log-transformed before statistical analysis and reported as geometric mean (\pm SD). IL-5 levels and blood and sputum eosinophils were not normally distributed and were therefore log-transformed prior to analysis and reported as median (range), for eosinophils, and geometric mean (\pm SD), for IL-5 levels. For between group analyses, analysis of variance (ANOVA) for repeated measures was applied. Within group changes in PC_{20} , sputum and blood eosinophils, serum and sputum supernatant IL-5 were analyzed using the Student's paired t-test. Mean differences between treatments with 95% confidence intervals for these effect estimates are presented for serum IL-5 and blood eosinophil counts. All statistical analyses were performed using the SPSS program. P-values < 0.05 were considered as statistically significant.

Results

Serum and sputum IL-5 levels

Serum IL-5 levels was rapidly cleared from the circulation and returned to baseline levels between 1 and 72 hours. Serum IL-5 concentrations in atopic asthmatics increased significantly 0.5 hours following intravenous IL-5 administration ($387.4 \pm 162.1\ \text{pg}/\text{ml}$), compared with baseline ($14.0 \pm 12.8\ \text{pg}/\text{ml}$, $p=0.002$), with rapid clearance, returning to baseline levels between 1 and 72 hours (Figure 2). The levels of serum IL-5 at 1, 2, 3, 4 and 5 hours after intravenous IL-5 were 16.8 ± 5.9 , 14.2 ± 8.4 , 5.7 ± 3.5 , 8.1 ± 3.9 , and $3.7 \pm 2.0\ \text{pg}/\text{ml}$ respectively, indicating that by one hour the levels had returned towards baseline values. In order to determine whether there was any difference in the kinetics

of clearance of IL-5 between asthmatic and non-asthmatic volunteers, we also studied 3 non-asthmatic non-atopic volunteers. After 2 µg intravenous injection, serum IL-5 measured in non-asthmatic subjects was 3.6 ± 2.5 pg/ml at baseline and increased to 54.2 ± 24.6 pg/ml 0.5 hours following intravenous IL-5 (mean difference -60.53 , 95% CI -107.7 - -13.3 , $p < 0.05$; Figure 2). These data indicate that there is no difference in the clearance in IL-5 between normal and asthmatic volunteers, although the asthmatics had higher levels of baseline serum IL-5 than non-asthmatics. There was a trend to elevation of serum IL-5 half an hour following inhaled IL-5 administration (18.18 ± 12.4 pg/ml to 51.14 ± 100.56 pg/ml) in the asthmatic subjects, but serum IL-5 was not affected by placebo. There was a significant increase in the IL-5 concentration measured in sputum supernatant from baseline at 4 and 24 hours following inhaled IL-5 (baseline: 49.0 ± 16.44 , 4 hours: $2,067 \pm 1,091$ and 24 hours: 152.3 ± 42.8 pg/ml; $p = 0.04$ and $p = 0.02$,

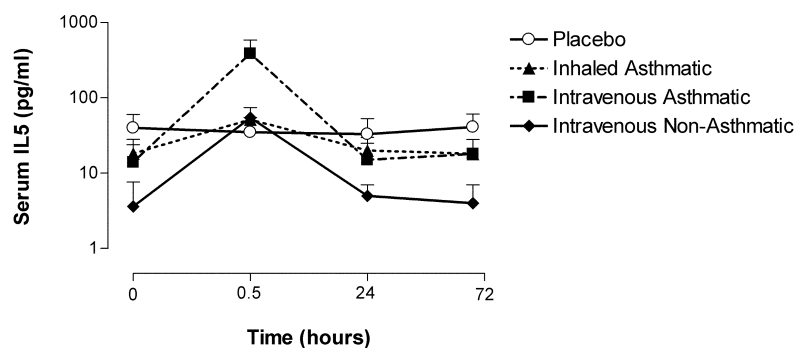


Figure 2A. Serum

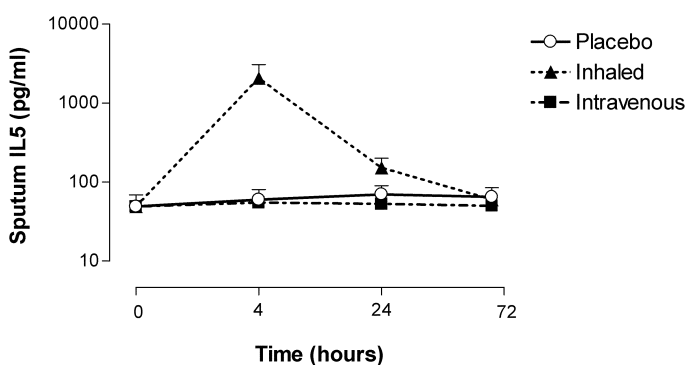


Figure 2B. Sputum

Figure 2.

- A. Mean IL-5 serum levels. Serum IL-5 levels rose markedly following intravenous IL-5 but were not significantly elevated by inhaled IL-5 or placebo in patients with mild asthma. In addition, in normal volunteers, the administration of intravenous IL-5 led to a peak of serum IL-5 levels at 0.5 hr similar to that in asthma patients.
- B. Mean IL-5 levels in sputum supernatants. Sputum IL-5 levels rose 4 hours following inhaled IL-5 and remained elevated at 24 hours but, were not affected by intravenous IL-5 or placebo.

respectively), but no changes in sputum supernatant IL-5 following placebo or intravenous IL-5.

Serum eotaxin

Serum eotaxin levels were 207.5 ± 80.2 ng/ml at baseline, 248.1 ± 93.6 at 0.5 hours, 232.5 ± 101 at 4 hours and 301.4 ± 113.6 at 24 hours following intravenous IL-5. There was a trend to increase in serum eotaxin following intravenous IL-5 with a $43.0 \pm 23.3\%$ increase at 24 hours compared to baseline (Figure 3). Comparative eotaxin levels following inhaled IL-5 was 12.0 ± 23.8 and after placebo 1.5 ± 6.8 . No correlation was observed between peak IL-5 levels (0.5 hours) and eotaxin levels at 0.5 or 24 hours following IL-5, nor was there correlation between eotaxin levels and peripheral blood lymphocyte or eosinophil numbers.

Effect of IL-5 on peripheral blood cells

Blood eosinophils were reduced 30 minutes (mean difference 0.15, 95% CI 0.05 – 0.26, $p < 0.01$) and 2 hours (0.13, 0.02 – 0.24, $p < 0.05$) following the administration of intravenous IL-5 compared with baseline (Figure 4). The decrease in eosinophils 0.5 hours after intravenous administration was significantly different from changes following inhaled IL-5 and placebo ($p < 0.03$). The increase in blood eosinophil counts observed at 3, 4, 5 and 72 hours was not statistically significant.

Additionally, peripheral blood lymphocytes were significantly increased from baseline at 4 ($p < 0.01$, mean difference -0.54 , 95% CI -0.97 - -0.11) and 5 hours ($p < 0.001$, -0.59 , -1.02 - -0.16) following intravenous but not inhaled IL-5 or placebo.

Effect of IL-5 on sputum eosinophils

The sputum eosinophil counts were 1.0% (0-6.3) (median (range)) at baseline. Following inhaled IL-5, sputum eosinophils increased to 5.0% (median) at 24 hours ($p = 0.02$), and persisted at 72 hrs (4.6%, $p = 0.02$). However, similar increases were observed after intravenous IL-5 and after placebo treatment (median at 24 hours: 3.6% and 3.7%, respectively, $p < 0.01$). There were no significant differences in sputum eosinophils between the treatment groups (Figure 5).

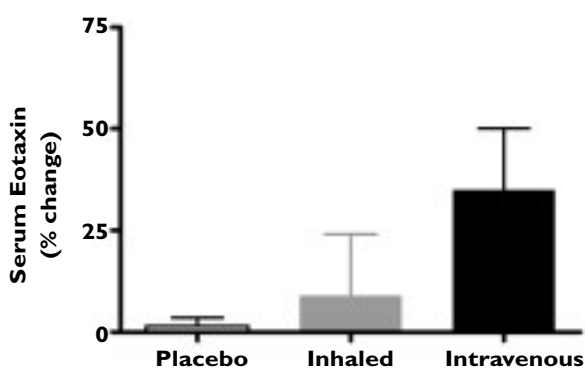


Figure 3. Serum eotaxin. There was a non-significant trend to increase in serum eotaxin levels 24 hours following intravenous IL-5 but not after placebo.

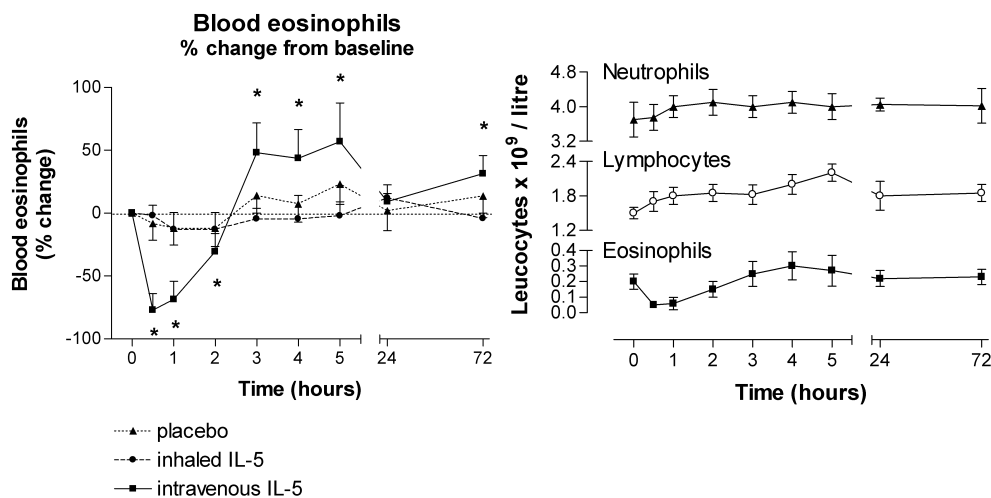


Figure 4A

Figure 4B

Figure 4

Left panel: Time course of blood eosinophil counts after placebo or inhaled IL-5 or intravenous IL-5. Intravenous IL-5 induced a rapid fall in circulating eosinophil numbers ($p < 0.05$), not seen following inhaled IL-5 or placebo. There was a prolonged but non-significant elevation of eosinophils at 3, 4, 5 and 72 hours after intravenous IL-5. Data shown as mean \pm SEM.

Right panel: Time course of changes in blood neutrophil, lymphocyte and eosinophil counts after intravenous IL-5. Data shown as mean \pm SEM.

Effect of IL-5 on lung function and bronchial responsiveness

Baseline FEV₁ was $95.1 \pm 3.8\%$ predicted, and PC₂₀ was 1.15 ± 2.21 mg/ml. No significant changes were observed in FEV₁ or PC₂₀ during the 3 different study periods (Figure 6). There were no significant correlations between sputum IL-5 levels and change in PC₂₀, nor between eosinophils in blood or sputum with levels of IL-5 measured in sputum or in serum.

Discussion

In this study, we report the first observations on the comparative effects of inhaled and intravenous IL-5 in mild asthma. We observed marked elevations of IL-5 levels in the serum but not in the sputum when administered intravenously, and the other way round when given by inhalation. Thus, inhaled IL-5 appeared confined to the lung and intravenous IL-5 to the blood compartment. Intravenous IL-5 induced a rapid decline and subsequent non-significant rise in blood eosinophils which was not associated with airway eosinophilia or bronchial hyperresponsiveness. Sputum eosinophils were indeed slightly increased after intravenous and inhaled IL-5 but were also observed following placebo, indicating that these increases were non-specific. Inhaled IL-5 had no systemic

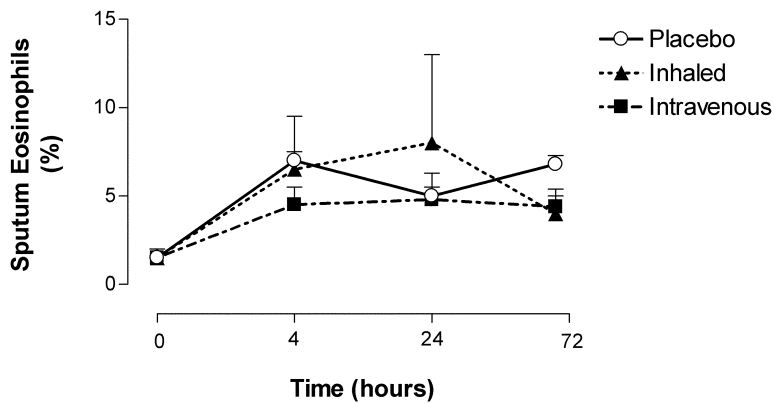


Figure 5. Mean sputum eosinophil counts. Sputum eosinophils were elevated four hours from baseline in each of the treatment groups, but no between-group differences occurred at any of the time points measured.

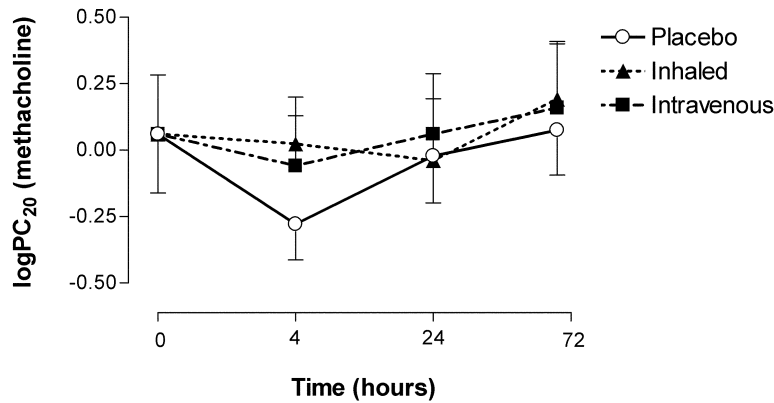


Figure 6. Mean log PC₂₀ to methacholine. Bronchial responsiveness did not change significantly from baseline after inhaled IL-5, intravenous IL-5 or placebo.

effect as reflected by blood eosinophils or serum eotaxin levels and did not affect bronchial responsiveness. A trend to elevated serum eotaxin levels was observed 24 hours following intravenous IL-5. The cellular source of this increase in eotaxin levels could have been the vascular endothelial cells, or circulating eosinophil and T-cells. Systemic IL-5 is a potent stimulus to eosinophil mobilisation to the circulation, albeit not in itself sufficient to specifically induce lung eosinophilia (as measured in induced sputum) or airway hyperresponsiveness.

We observed significant, sequential and converse effects on peripheral blood eosinophils following intravenous IL-5. Between 0.5 and 2 hours, eosinophils were markedly reduced, and this effect has not been previously reported in animals or humans. At later time-points (4-72 hours) there was significant elevation of blood eosinophil counts. We speculate that these effects result from rapid vascular margination followed by, either

demargination or replenishment of the circulating eosinophil pool from bone marrow, and other extra-medullary leukocyte reservoirs. A rapidly-induced but short-lived peripheral eosinophilia has been observed previously following intravenous IL-5 in animals (20;21), while a prolonged peripheral blood eosinophilia occurred after inhaled IL-5 in human asthmatics (13).

That IL-5 did not specifically affect sputum eosinophils levels contradicts a previous study of IL-5 inhalation in mild asthmatics (14). We observed minor but significant increases in sputum eosinophil numbers in all groups. These changes are unlikely to be explained by methacholine challenge (22) or repeated sputum induction (23). Our findings are, however, consistent with previous studies in rodents. Wang et al (1998) (24) demonstrated that circulating IL-5 is essential for the development of airway eosinophilia, whereas local lung IL-5 production did not lead to eosinophil recruitment to the lungs. Similarly, Mould et al (2000) (11) observed a doubling of eosinophil counts in bronchoalveolar lavage fluid following the administration of intrapulmonary and intravenous IL-5 compared to intrapulmonary IL-5 administration alone in mice. Lee and coworkers (1997) created an IL-5 transgenic mouse expressing IL-5 in airway epithelium which developed baseline bronchial hyperresponsiveness and BAL eosinophilia; however, in this model IL-5 was expressed at unusually high levels in serum with concomitant prominent peripheral eosinophilia (12).

We saw no effect of IL-5 on bronchial responsiveness. Consistent findings are provided in human studies in which monoclonal anti-IL-5 blocking antibodies substantially reduced allergen-induced blood and sputum eosinophilia but had no effect on the late phase response and bronchial hyperresponsiveness to allergen challenge (25). These findings have been consistently confirmed in animal models (26;27). The contribution of IL-5 to bronchial hyperresponsiveness is contentious and in asthma models, difficult to dissociate from effects induced by mediators other than IL-5 (28-31). Consistently however, IL-5 transgenic mice have prominent airway eosinophilia but have acetylcholine responses similar to wild type mice (32). These studies collectively suggest a close association between IL-5 and both circulating and bronchial eosinophil numbers but a dissociation between pulmonary IL-5 and bronchial responsiveness in both animals and humans (25; 31-33).

Our observations are substantially different from those of Shi et al (14) in terms of sputum eosinophilia and bronchial responsiveness induced by IL-5, despite using similar amounts of inhaled IL-5 obtained from a similar source, and comparable cohorts of asthmatics in terms of severity and baseline sputum eosinophilia. These studies were performed in ethnically different populations raising the possibility that there may be racial susceptibility to the effects of IL-5. Another possibility is that the high level of endemic parasitic colonization in China (34-36) may induce an expanded population of activated tissue eosinophils(35-37), that are primed to respond more to IL-5. Sputum ECP levels in the mild asthmatics studied by Shi et al were, however, 2-log fold higher than levels previously described in severe asthma (38).

Collins et al 1995 (9) reported that in contrast with intravenous administration, local IL-5 did not induce tissue accumulation of eosinophils (9). This indicates that local IL-5 has little chemoattractant activity, whereas intravenous IL-5 has a rapid enhancing effect on eosinophil accumulation, by stimulating the release of a rapidly mobilizable pool of bone marrow eosinophils. Furthermore, although IL-5 is necessary, other signals from activated T lymphocytes may also be required to induce accumulation of eosinophils in the airways (20;39;40). Eotaxin, an eosinophil selective chemokine, augments the accumulation of eosinophils by IL-5 in a synergistic fashion. This implies that IL-5 and eotaxin act co-operatively to promote the recruitment of eosinophils into the tissue (9;20) Our data indicates that IL-5 when administered on its own to the airways by aerosol is not capable of inducing eosinophilic inflammation and bronchial hyperresponsiveness in mild asthmatic subjects. However, changes in circulating eosinophil numbers were observed with intravenous administration of IL-5, indicating potential systemic effects on the bone marrow. Thus, IL-5 may have systemic rather than pulmonary effects in asthma.

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