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Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics

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Increases in airway eosinophils and interleukin-5 with minimal bronchoconstriction during repeated low-dose allergen challenge in atopic asthmatics. I. Sulakvelidze, M.D. Inman, T. Rerecich, P.M. O'Byrne. ©ERS Journals Ltd 1998.

ABSTRACT: Repeated low-dose allergen challenge increases airway hyperresponsiveness in atopic asthmatics. However, it is not known whether low-dose allergen challenge increases airway inflammation.

Eight atopic asthmatics were enrolled in a controlled, cross-over study to evaluate the effect and time course of repeated low-dose allergen challenge on airway inflammation and hyperresponsiveness. The dose of allergen to reduce forced expiratory volume in one second (FEV_1) by approximately 5% was selected in a screening allergen challenge. The subjects then were challenged for five consecutive days with either diluent or the selected low-dose of allergen. Methacholine airway hyperresponsiveness ($PC_{20,meth}$) was measured and sputum induced on days 1, 3 and 5 of the repeated challenge, and then 1 day and 3 days after the last challenge.

Repeated low-dose allergen challenge caused small reductions in FEV_1, but increased airway eosinophils and interleukin (IL)-5, airway hyperresponsiveness, asthma symptoms and β_2-agonist use, all of which peaked on days 3 or 5 of the challenge. The mean (SEM) percentage sputum eosinophils was 21.2 (0.7)% after allergen *versus* 3.9 (0.1)% after diluent ($p<0.001$); percentage EG2+ cells were 13.4 (0.3)% after allergen *versus* 1.1 (0.04)% after diluent ($p<0.01$) and geometric mean (GSEM) eosinophil cationic protein (ECP) was 1061.8 (1.6) $\mu g \cdot L^{-1}$ after allergen *versus* 447.03 (1.2) $\mu g \cdot L^{-1}$ after diluent ($p<0.05$). Geometric mean (GSEM) IL-5 was 71.4 (1.4) $pg \cdot mL^{-1}$ after allergen *versus* 18.4 (1.04) $pg \cdot mL^{-1}$ after diluent ($p<0.01$). All the changes had resolved by 3 days after the last challenges.

The study demonstrated that repeated inhalation of a low-dose of allergen causes airway eosinophilia and increases in interleukin-5, associated with airway hyperresponsiveness, and mild worsening of asthma control, without the development of marked acute bronchoconstriction or the development of late responses.

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Asthma induced by the inhalation of various allergens in the clinical research laboratory, has been a model for studying pathophysiology and pathogenesis of this disease for many years [1–4]. The inhalation of a specific allergen by asthmatic subjects can cause three types of airway responses [5]: the isolated response, that develops within 10–30 min after allergen challenge; the isolated late response that develops 3–8 h after the challenge; and the dual response, where subjects develop both early and late airway responses. The late response is associated with allergen-induced increases in airway responsiveness [6] and with eosinophilic airway inflammation [7].

The doses of allergen usually used to study experimentally induced asthma are of a magnitude that causes an early response of at least a 15% reduction in forced expiratory volume in one second (FEV_1) and a late response of at least 20% [5]. A shortcoming of this method is that these doses of allergen may be much higher than those to which patients with asthma are exposed in their natural environments. This shortcoming has been addressed by IHRE and co-workers [8, 9], who used airway challenge with a low-dose of allergen. At first, these investigators studied early and late responses after a single challenge with low-dose allergen [8]. Later, they used repeated low-dose allergen challenge and demonstrated a significant increase in airway hyperresponsiveness [9]. However, the role of airway inflammation in causing these changes, as well as the time course of these events, has not been studied previously.

The purposes of the present study were: 1) to determine the effects of repeated low-dose allergen challenge on airway inflammation as measured by numbers of eosinophils and metachromatic cells in induced sputum, as well as markers of eosinophil activation and level of the eosinopoietic cytokine IL-5; 2) to evaluate the effects of repeated low-dose allergen challenge on the development of late responses and methacholine airway hyperresponsiveness and to examine the time course of the changes; and 3) to evaluate the effects of repeated low-dose challenge on asthma symptoms and β_2-agonist use.

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Table 1. – Subject characteristics at the screening visit

Sex	Age	Allergen type	Screening allergen* (dilution)	Low-dose allergen+ (dilution)	FEV_1 % pred	$PC_{20,meth}$ mg·mL^{-1}
M	26	Cat	1:256	1:1024	89.7	0.8
M	22	HDM	1:1024	1:8192	81.2	1.9
M	45	HDM	1:8	1:64	80.6	10.6
F	23	Ragweed	1:4	1:64	112.1	8.0
M	20	Ragweed	1:256	1:1024	93.1	3.5
M	22	Ragweed	1:16	1:256	98.6	1.3
F	19	HDM	1:256	1:512	93.2	1.1
M	21	HDM	1:4	1:64	90.1	3.0

*: top concentration of allergen used for allergen challenge producing an early asthmatic response of a 20% fall in FEV_1; +: concentration of allergen used for repeated low-dose inhalations. FEV_1: forced expiratory volume in one second; $PC_{20,meth}$: provocative concentration of methacholine causing a 20% fall in FEV_1; M: male; F: female; HDM: house dust mite.

Methods

Subjects

Ten nonsmoking subjects with mild allergic asthma were recruited for the study (seven males, three females) (table 1). All subjects had had mild asthma symptoms for more than a year, that required only occasional use of β_2-agonists. They had no exacerbations of asthma or respiratory tract infections for at least 4 weeks before entering the study. Only subjects who had demonstrated a dual airway response during a screening allergen challenge were recruited. The subjects were asked to withhold β_2-agonists and caffeine containing beverages for 7–8 h before challenge tests. The study protocol was approved by the Ethics Committee of McMaster University Health Sciences Center and all subjects gave written informed consent to participate in the study. Only eight subjects completed the study. One subject was excluded because of inability to produce sputum, and one because of an exacerbation of asthma after the screening allergen challenge.

Study design

A cross-over study, comparing repeated allergen challenge with the allergen diluent was used to study the effects of repeated low-dose allergen challenge on airway inflammation and responsiveness. The primary outcome of the study was a change in sputum eosinophils. The secondary outcomes were numbers of sputum activated eosinophils (EG2+ cells), levels of eosinophil cationic protein (ECP) and IL-5, as well as metachromatic cells, blood eosinophils, methacholine airway responsiveness, FEV_1, asthma symptoms and the use of β_2-agonists. The study was not randomized. In all subjects, diluent challenge was administered first, followed by the allergen challenge. Sub-jects were not randomly assigned to the allergen or diluent challenge because of uncertainty about the duration of the washout period needed to eliminate the consequences of the repeated inhalations of allergen. However, physiological and all sputum measurements were made by an investigator who was blind to the identity of the subjects.

Subjects were initially challenged with the diluent on five sequential weekdays, in the morning between 08:00 and 10:00 h. FEV_1 measurements were taken before and every 10 min after the challenge, for 30 min. On three weekdays, every other day, the subjects made a second visit to the laboratory between 14:00 and 16:00 h. During the second visit, they were challenged with methacholine and sputum was induced. One day and 3 days after the last repeated challenges subjects again visited the laboratory for methacholine challenge and sputum induction. At each visit subjects were asked to answer a questionnaire dealing with their asthma symptoms and use of β_2-agonists during the previous day. After 1 week washout, the subjects were crossed over and the same protocol was repeated, this time with the low-dose allergen challenge.

When selecting the appropriate low-dose of allergen to be used in the repeated challenge, we had two major objectives. Firstly, the dose should be significantly lower than that used in a conventional allergen challenge. Secondly, it should be large enough to induce small, but measurable, change in FEV_1 to ensure that the subject did, in fact, respond to the allergen. A dose of allergen causing a fall in FEV_1 of approximately 5% satisfied both requirements. This low-dose was estimated during the screening visit when subjects were given conventional allergen challenge (table 1).

Methacholine challenge test

Methacholine challenge tests were performed using the method of COCKCROFT *et al.* [10]. Subjects first inhaled normal saline followed by increasing doubling concen-trations of methacholine until FEV_1 fell by 20% or more from the baseline. Concentrations of methacholine were log-transformed and the concentration causing a 20% fall in FEV_1 ($PC_{20,meth}$) was calculated using a formula given by JUNIPER *et al.* [11].

Conventional allergen challenge test

Allergen challenge tests were performed according to the method described by O'BYRNE *et al.* [5]. Based on the size of the allergy skin test and $PC_{20,meth}$, the concentration of allergen that would cause a fall in FEV_1 of 20% in each individual subject was calculated [12]. The challenge was started with a dose that was two doubling concentrations lower than the calculated dose. FEV_1 was measured before and 10 min and 20 min after the challenge. If FEV_1 did not fall by 20% or more, increasing doubling concentrations of allergen were administered until FEV_1 had fallen by 20% or more from the baseline. FEV_1 was then monitored at 30, 40, 50, 60, 90 and 120 min and then every hour, until 7 h after the challenge.

Repeated low-dose allergen challenge

A dose of allergen that caused a 5% fall in FEV1 was determined during the screening allergen challenge. This dose was administered as a single challenge on the early mornings of five consecutive weekdays. Normal saline inhalations were administered in the early mornings of the control week.

Sputum induction and analysis

Sputum was induced using the method described by PIN *et al*. [13], by inhalation of a hypertonic saline. Concentrations of 3, 4 and 5% were inhaled for 7 min or until FEV1 dropped by 20%. Salbutamol (200 μg) was given 10 min prior to sputum induction and FEV1 was measured before and after the procedure. If enough sputum was ob-tained with any concentration of hypertonic saline, the challenge was discontinued. Plugs were selected from the obtained sputum sample and treated with 0.1% dithioth-reitol (Sputolysin; Calbiochem-Behring, San Diego, CA, USA) and Dulbecco's phosphate buffered saline (Gibco Diagnostics, Tucson, AZ, USA). Cytospins were then prepared on glass slides for histo- and immunohistochemical staining [14]. For differential cell count, slides were stain-ed with Diff-Quik (American Scientific Products, McGaw Park, IL, USA) and 400 cells were counted under the light microscope. For methachromatic cell counts, slides were stained with Toluidine Blue and 5,000 cells were counted. Activated eosinophils were detected through immunohistochemical staining of aptex-coated slides using a monoclonal EG2 antibody (Kabi Pharmacia, Uppsala, Sweden) directed against cleaved intracellular ECP. Sputum IL-5 was measured using enzyme-linked immunosorbent assay (ELISA) (R&D, Minneapolis, MN, USA) and ECP was measured using radioimmunoassay (RIA) (Kabi Pharmacia, Uppsala, Sweden).

Asthma symptoms and β_2-agonist use

On the morning of each study day, subjects were given a questionnaire to report their daytime and night-time symptoms as well as use of β_2-agonists during the previous day. A four point grading scale for symptoms was used: 0=absence of symptoms; 1=mild symptoms; 2=moderate; and 3=severe symptoms. Use of β_2-agonists was expressed as the number of puffs. Oral prednisone tablets were dispensed to all subjects before the start of the study and a detailed written asthma self-management protocol was discussed and given to the subjects in the event of an asthma exacerbation during the study.

Statistical analysis

The primary outcome of the study was sputum eosinophils. Sample size was estimated based on our previous study of airway inflammation after conventional allergen challenge. An increase of 15% in sputum eosinophilia, dur-ing repeated allergen challenge, with a significance level of p=0.05 and power of 80% required a study population of nine subjects. Secondary outcomes of the study were sputum EG2+ eosinophils, ECP and IL-5, sputum metachromatic cells, blood eosinophils, $PC_{20,meth}$, FEV1, as-thma symptoms and use of inhaled β_2-agonists. $PC_{20,meth}$ data were log-transformed for analysis and are reported as geometric means (GSEM). Sputum eosinophils and EG2+ eosinophils were expressed as percentages and square root transformed for statistical analysis [15]. Sputum metachromatic cells were expressed as absolute numbers per 5,000 total counted cells, and square root transformed for analysis. However, the values reported for eosinophils, EG2+ eosinophils and metachromatic cells throughout the text represent anti-square root transformed means (anti-square root transformed SEM). Sputum ECP and IL-5 were log-transformed for statistical analysis; however, these variables are reported (analogous to $PC_{20,meth}$) as geome-tric means (GSEM). Two-factor repeated measures analysis of variance (ANOVA) was used to analyse normalized data. The two independent variables were challenge type (diluent or allergen) and time (challenge day). Dependent variables have been already described in detail. Significance was accepted at the level of p=0.05 and multiple comparisons were performed by Student-Neuwman-Kuels procedure.

Results

The dilutions of allergen used in the repeated challenges were between two and 16 fold lower than those used in the initial screening allergen challenges, with a median value of eightfold less (table 1).

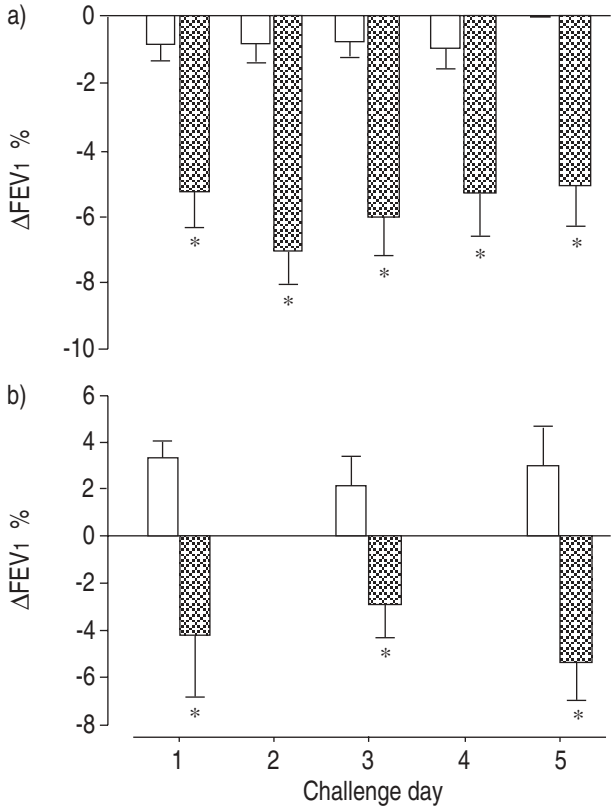

Fig. 1. – Mean (±SEM) maximal early (0–30 min) (panel a) and late (7 h) (panel b) change in forced expiratory volume in one second (ΔFEV1) after repeated inhalation of diluent (▭) or low-dose allergen (▩). The late responses were measured on days 1, 3 and 5 of the repeated challenges. Repeated low-dose allergen challenge caused a small, but significant, fall in FEV1 both early and late after challenge. *: p<0.05.

The mean (±SEM) maximal immediate fall in FEV1 after low-dose allergen challenge was 7.01±1.02%, compared to 0.9±.0.6% after diluent (fig. 1). The fall in FEV1 on each challenge day was significantly greater after low-dose allergen challenge compared to diluent ($p<0.01$). The mean (±SEM) maximal fall in FEV1 measured 7 h after allergen challenge was 5.3±1.6% compared to an increase in FEV1 of 3.1±1.5% after diluent, which was achieved on day 5 ($p<0.01$) (fig. 1).

Repeated low-dose allergen challenge significantly increased night-time asthma symptoms ($p<0.01$), and night-time β_2-agonist use ($p<0.01$) (fig. 2). Significant increase in symptoms occurred on days 2, 3, 4 and 5. The increase in β_2-agonist use was only significant on day 5. There was no significant difference in night-time symptoms and β_2-agonist use between the allergen and placebo 1 day after the challenge.

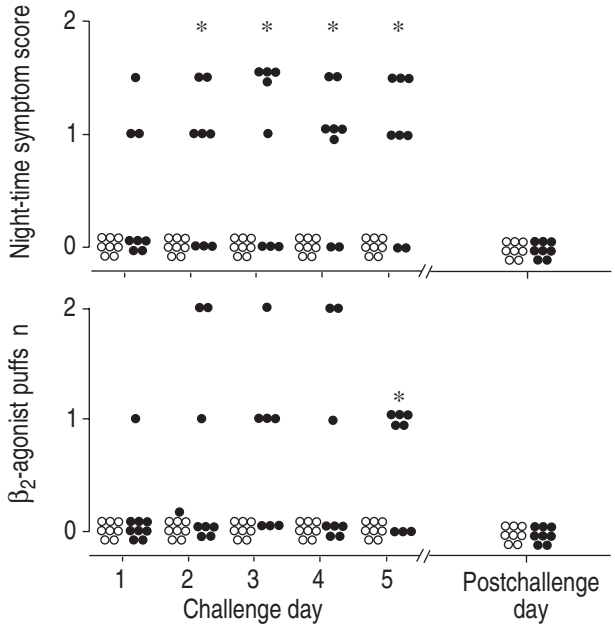

Fig. 2. – Nocturnal asthma symptoms and β_2-agonist use during repeated diluent (○) or low-dose allergen (●) challenge. The repeated allergen challenges caused a significant increase in asthma symptoms and β_2-agonist use. These increases had resolved by 1 day after the repeated challenges. *: $p<0.05$.

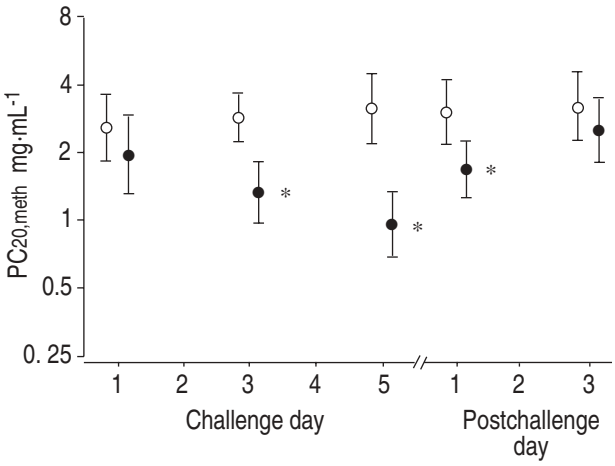

Fig. 3. – Provocative concentration of methacholine causing a 20% fall in forced expiratory volume in one second ($PC_{20,meth}$) values (mean and GSEM) after repeated inhalation of diluent (○) or low-dose allergen (●). Repeated low-dose allergen challenge caused a significant reduction in $PC_{20,meth}$, which had resolved by day 3 after the challenges. *: $p<0.05$.

Repeated low-dose allergen challenge significantly reduced $PC_{20,meth}$ (fig. 3). The effect was most marked on day 3, where the geometric mean $PC_{20,meth}$ was 1.3 mg·mL^{-1} (GSEM 1.4) after allergen compared to 2.8 (1.3) mg·mL^{-1} after diluent ($p<0.01$) and day 5, where the mean $PC_{20,meth}$ was 0.96 (1.4) mg·mL^{-1} after allergen compared to 3.2 (1.4) mg·mL^{-1} after diluent ($p<0.01$). A significant difference between the allergen and diluent periods was still present 1 day after the last challenges, when the mean $PC_{20,meth}$ was 1.7 (1.3) mg·mL^{-1} after allergen compared to 3.03 (1.4) mg·mL^{-1} after diluent ($p<0.01$). However, the effect had resolved by 3 days after the challenges, when the mean $PC_{20,meth}$ was 2.5 (1.4) mg·mL^{-1} after allergen compared to 3.2 (1.4) mg·mL^{-1} after diluent ($p=0.5$).

Repeated low-dose allergen challenge significantly increased sputum eosinophils, the increases being most marked on day 3, when the mean (SEM) value was 17.8 (0.4)% after allergen compared to 3.9 (0.1)% after diluent ($p< 0.001$), and day 5, where the mean value was 21.2 (0.7) % after allergen compared to 3.9 (0.1)% after diluent ($p< 0.001$) (fig. 4). However, no significant differences were present between the allergen and diluent periods 1 day after the last challenge.

EG2+ stained eosinophils also increased significantly during repeated low-dose allergen challenge, being most marked on day 3 where the mean value was 6.1 (0.22)% after allergen compared to 1.8 (0.02)% after diluent ($p< 0.05$) and day 5, where the mean value was 13.4 (0.3)% after allergen compared to 1.1 (0.04)% after diluent ($p< 0.01$) (fig. 4). However, no significant differences were present in EG2+ cells between allergen and diluent periods 1 day after the last challenge.

Sputum ECP level was significantly higher during repeated low-dose allergen challenge than during diluent

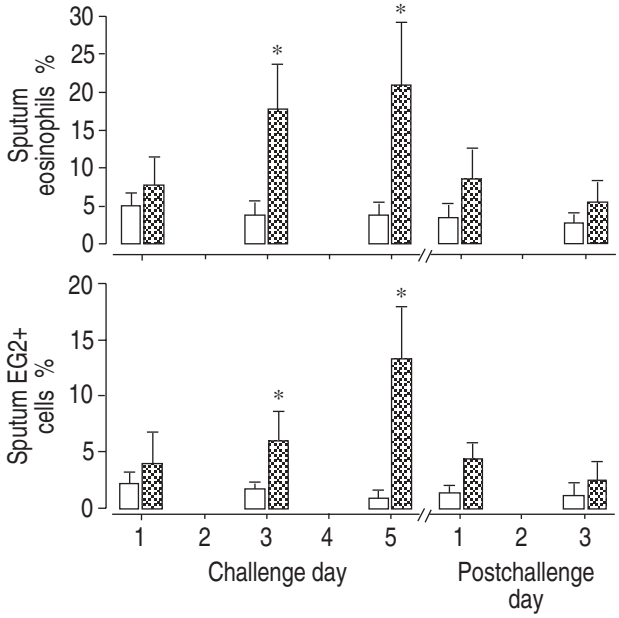

Fig. 4. – Changes in a) sputum eosinophils and b) EG2+ cells after repeated inhalation of diluent (▭) or low-dose allergen (▩). Repeated low-dose allergen challenge caused a significant increase in both eosinophils and EG2+ cells, which were maximal by day 5 of the challenges, and were no longer significantly increased by 1 day after the challenges. *: $p<0.05$.

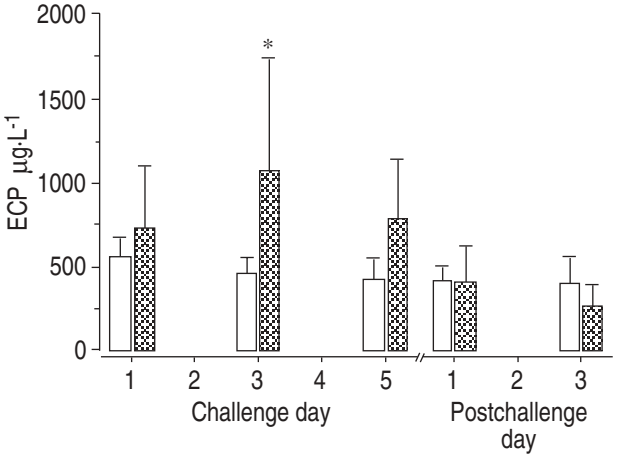

Fig. 5. – Changes in sputum eosinophil cationic protein (ECP) after repeated inhalation of diluent (▭) or low-dose allergen (▩). Repeated low-dose allergen challenge caused a significant increase in sputum ECP, which was only significant on day 3 of the challenges. *: p<0.05.

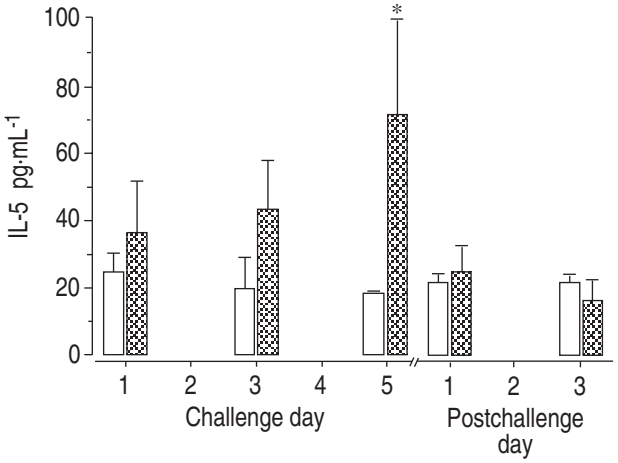

Fig. 6. – Changes in sputum interleukin (IL)-5 after repeated inhalation of diluent (▭) or low-dose allergen (▩). Repeated low-dose allergen challenge caused a significant increase in sputum IL-5, which was only significant on day 5 of the challenges. *: p<0.05.

challenge. The difference peaked on day 3 where the geometric mean value (GSEM) after allergen was 1061.8 (1.6) µg·L^{-1} compared to 447.03 (1.2) µg·L^{-1} after diluent (p< 0.05) (fig. 5). However, no significant difference was noted between the allergen and diluent periods 1 day after the last challenge.

During the diluent challenge period sputum IL-5 was either at the lowest measurable level or undetectable in the majority of subjects. However, during the low-dose allergen challenge period it became detectable in all subjects except one. The difference in IL-5 between the allergen and diluent challenges peaked on day 5, the geometric mean value (GSEM) being 71.4 (1.4) pg·mL^{-1} after allergen compared to 18.4 (1.04) pg·mL^{-1} after diluent (p<0.01) (fig. 6). No differences were seen between allergen and diluent phases 1 day after the last challenges.

Sputum metachromatic cells increased significantly during repeated low dose allergen challenge. The greatest increases occurred on days 3 and 5, and metachromatic cells were still significantly increased 1 day, but not 3 days, after the last challenges. On day 3 the mean value (SEM) per

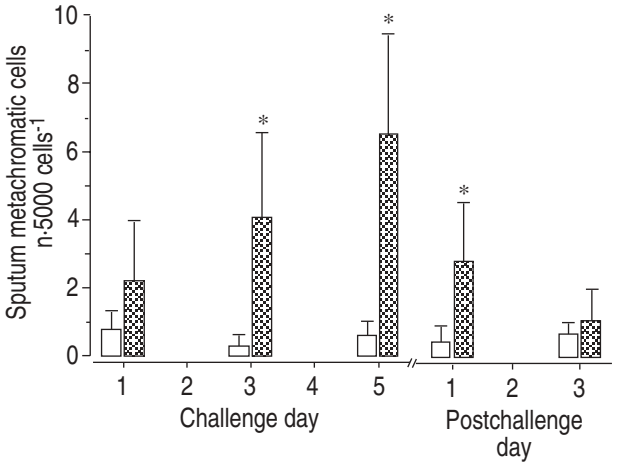

Fig. 7. – Changes in sputum metachromatic cells after repeated inhalation of diluent (▭) or low-dose allergen (▩). Repeated low-dose allergen challenge caused a significant increase in sputum metachromatic cells, which had resolved by day 3 after the challenges. *: p<0.05.

5,000 cells was 4.0 (0.3) after allergen compared to 0.23 (0.1) after diluent (p<0.01) and on day 5, the mean value was 6.49 (0.27) after allergen compared to 0.53 (0.05) after diluent (p<0.001) (fig. 7). One day after allergen the mean value was 2.75 (0.21) compared to 0.4 (0.07) after diluent (p<0.01).

Blood eosinophils overall were significantly increased only 1 day after the last of the repeated challenges, the mean value being 331.2±63.2 after allergen compared to 218.7±46.2 after diluent (p=0.04).

Discussion

This study has shown that repeated low-dose allergen challenge causes airway inflammation. This was demonstrated by significant increases in sputum eosinophils, EG2+ eosinophils and sputum supernatant ECP, as well as metachromatic cells on days 3 and 5 of allergen challenge compared to diluent challenge; however, these differences were no longer significant at 1 or 3 days after the last challenge. Sputum IL-5 also increased during the whole period of repeated allergen challenge compared to diluent; however, the peak level was reached on day 5. The study has also shown that repeated low-dose allergen challenge significantly increased airway responsiveness as early as day 3 of the challenge. This increase was still present at 1 day, but not 3 days, after the last challenge. Finally, repeated low-dose allergen challenge caused a slight, but significant, increase in nocturnal asthma symptoms and β_2-agonist use. These effects were obtained with concentrations of allergen that were a median eightfold lower than that used in conventional allergen challenges, and which were not associated with marked acute bronchoconstriction or the development of conventional late responses of a greater than 15% fall in FEV1.

The increase in sputum eosinophils and sputum EG2+ cells after repeated low-dose allergen challenge peaked on day 5 of the challenge and was associated with the largest changes in methacholine PC20,meth. The magnitude of the increase in sputum eosinophils is similar to that found by other investigators in sputum or bronchoalveolar lavage (BAL) after conventional allergen challenge [7, 16–18].

Interestingly, the changes in sputum eosinophils, EG2+ cells and methacholine airway hyperresponsiveness were accompanied by an increase in nocturnal asthma symptoms, as well as β_2-agonist use. These results are in agreement with other studies that have suggested the functional importance of activated eosinophils in asthma [19, 20].

Our finding of increased sputum ECP during repeated low-dose allergen challenge is in agreement with those studies that have shown increase in sputum ECP in stable asthma, as well as during exacerbations [21, 22]. In our study, sputum ECP peaked on day 3 of the repeated challenge, whereas the percentage of EG2-positive eosinophils peaked on day 5. These findings may mean that eosinophils contribute to the pathophysiological changes that occur in the later phase of the repeated low-dose allergen challenge through mechanisms other than secretion of ECP.

An important role has been ascribed to IL-5 in the pathogenesis of asthma by several investigators [23–25]. The present study has demonstrated an increase in IL-5 that was observed on the first day of allergen challenge, but only reached statistical significance on day 5. These results are consistent with an important role for IL-5 in providing an appropriate environment for the maturation and prolonged survival of eosinophils newly recruited into the airways during repeated low-dose allergen challenge, and support an important role for IL-5 in this regard. The study has not identified the predominant source of IL-5, but parallel patterns of increase of IL-5 and eosinophils imply the possibility of a bidirectional interaction between them [25–27].

Sputum metachromatic cells, in common with eosinophils, significantly increased on day 3 and peaked on day 5 of repeated low-dose allergen challenge. However, unlike eosinophils, sputum metachromatic cells remained significantly increased on day 1 after the last challenge. The pattern of change in sputum metachromatic cells closely followed that of methacholine $PC_{20,meth}$. There is evidence in the literature implicating metachromatic cells in the pathogenesis of asthma. PIN *et al*. [17] and PIZZICHINI *et al*. [28] demonstrated an increase in sputum metachromatic cells after allergen challenge or during the nat-ural course of disease. Furthermore, metachromatic cells obtained by bronchial brushings have been shown to correlate significantly with $PC_{20,meth}$ in asthma [29] and the mast cell-derived mediators histamine and tryptase have been increased in BAL in patients with symptomatic asthma [30].

There are other pathways, in addition to IL-5, that may have lead to the marked eosinophilia following repeated low-dose allergen challenge. One of these may be leukotriene dependent. Leukotrienes do have the capacity to attract eosinophils to the airways [31], in addition to playing an important role in allergen-induced early and late asthmatic responses [32]. Repeated low-dose allergen challenge may be an appropriate clinical model for sorting out the relative contribution of IL-5 and leukotrienes to asthmatic airway inflammation.

This study revealed rather fast resolution of the airway inflammation caused by repeated low-dose allergen challenge; all inflammatory indices returned to the baseline by 3 days after the last challenge. This suggests that endogenous anti-inflammatory mechanism(s) may be involved in resolving the inflammation, in an effort to minimize the tissue-damaging effects of repeated allergen challenge. However, the precise mechanism of this resolution was not evaluated in this study.

The pattern of allergen inhalation during the study was designed to mimic the natural course of allergic asthma more closely than the conventional allergen inhalation challenge. Similar increases in airway hyperresponsiveness and airway inflammation have been described by several other groups who have studied these indices during natural pollen exposure [7, 33]. The experimental approach of using conventional allergen challenge to study pathophysiology of asthma has been criticized [34]. The main reason for this criticism is the fact that allergen challenge involved brief single exposure to a high dose of allergen, which would not often occur in the everyday life of the majority of asthmatics. The use of repeated low-dose challenge does not fully get around this criticism, as the allergen is inhaled only once throughout the day, while natural allergen exposure is likely to be of even lower dose, and more prolonged. This study does demonstrate, however, that repeated doses of inhaled allergens, which cause very mild degrees of bronchoconstriction, can cause airway inflammation, airway hyperresponsiveness and slight deterioration of asthma control. We had initially postulated that, should this occur, the inhalation of the allergen on days 4 and 5 may be associated with more marked acute bronchoconstriction immediately after the challenge, or the development of late responses. However, the only progressive physiological abnormality that we could measure was the increase in airway hyperresponsiveness. This suggests that changes of methacholine airway hyperresponsiveness may be more sensitive to changes in the inflammatory status of the airways than late responses.

The potential advantages of repeated low-dose allergen challenge over conventional allergen challenge are not only the greater clinical relevance of the methods of challenge, but also the safety of the challenge, where no major bronchoconstrictor response is induced.

However, the repeated challenge is a major commitment for the subjects involved in the study, where 10 laboratory visits, of approximately 1 h each, are required for each of the allergen and diluent in each part of the study. This compares to three laboratory visits for each part of a conventional allergen challenge; however, if a late response is being evaluated, the time commitment is also approximately 10 h. Also, in some laboratories, subjects are kept in hospital on the night of allergen challenge, because of concerns about development of severe late responses. This is unnecessary when a repeated low-dose challenge is performed.

The repeated challenge has provided somewhat different information with regards to allergen-induced airway inflammation, when compared to the conventional challenge. For example, there were no significant differences in any of the studied indices between allergen and placebo, at 7 h after the first of the repeated low-dose challenges. By contrast, the conventional challenge is accompanied by increased numbers of sputum eosinophils and metachromatic cells, and by airway hyperresponsiveness 7 h after the challenge [6, 35]. Furthermore, we could not find significant differences in any of the physiological or inflammatory indices at 3 days after the last repeated allergen challenge. However, the changes caused by conventional allergen challenge last longer than 3 days [6].

In conclusion, this study has shown that repeated low-dose allergen challenge causes an increase in sputum eosinophils, activated eosinophils, eosinophil cationic protein and interleukin-5, as well as metachromatic cells. All of these inflammatory changes resolve by 3 days after the last allergen challenge. Also, repeated low-dose allergen challenge increases methacholine airway responsiveness, which persists for 1 day, but has resolved by 3 days after the last challenge. The repeated challenge is accompanied by a small but significant reduction in forced expiratory volume in one second, as well as an increase in asthma symptoms and use of β_2-agonists.

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