

Effects of once daily dosing with inhaled budesonide on airway hyperresponsiveness and airway inflammation following repeated low-dose allergen challenge in atopic asthmatics

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Summary

Background Repeated low-dose allergen challenge increases airway hyperresponsiveness and sputum eosinophils in atopic asthmatics. Inhaled corticosteroids attenuate the airway responses to high-dose allergen challenge, but have not been evaluated against repeated low dose challenge.

Objective This study evaluates the effects of once daily treatments of two doses of inhaled budesonide on airway responses to repeated low-dose allergen challenge.

Methods Eight atopic asthmatics with a dual airway responses to inhaled allergen were recruited into a randomized, double-blind crossover, placebo-controlled study. In the mornings of four consecutive days (day 1–day 4), subjects inhaled budesonide 100 µg, 400 µg, or placebo, 30 min before inhaling a concentration of allergen causing a 5% early fall in FEV₁. Airway hyperresponsiveness to methacholine and sputum eosinophils were measured at baseline, on the afternoon of day 2, day 4, and 24 h after the last challenge. There was a 1-week washout between each of the three treatment periods.

Results The repeated low-dose allergen challenge induced increases in the percentage sputum eosinophils from $2.0 \pm 0.7\%$ at baseline to $16.6 \pm 7.1\%$ on day 4 ($P = 0.002$), and this effect was reduced by once daily budesonide 100 µg to $5.6 \pm 1.8\%$ ($P = 0.01$) and by once daily budesonide 400 µg to $3.1 \pm 0.9\%$ ($P = 0.004$). Also, the allergen-induced methacholine airway hyperresponsiveness which occurred by day 4 ($P = 0.03$) of the repeated low dose challenge was inhibited by budesonide 400 µg ($P = 0.017$).

Conclusion Both budesonide 100 µg and 400 µg inhaled once daily significantly reduces allergen-induced sputum eosinophilia after repeated low dose challenge; however, only the higher dose also attenuates the allergen-induced airway hyperresponsiveness.

Keywords: low-dose allergen challenge, glucocorticosteroid, airway hyperresponsiveness, airway inflammation.

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Introduction

Inhalation of relevant allergens by atopic asthmatics is known to cause an asthmatic response. Subjects who

develop allergen-induced late bronchoconstrictor responses after high-dose allergen challenge also develop airway hyperresponsiveness to bronchoconstrictor agonists, such as methacholine and histamine [1,2], and increased inflammatory cells, particularly eosinophils and metachromatic cells in airway secretions [3,4]. Repeated low-dose allergen inhalation challenge, which causes minimal bronchoconstriction, has been utilized to mimic more

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long-term environmental exposure to seasonal allergens, and results in increased airway hyperresponsiveness [5–7] which is also observed during seasonal allergen exposure [8]. Recently, repeated low-dose allergen inhalation in subjects who develop late allergen-induced bronchoconstrictor responses to high-dose allergen challenge, has been shown to result in enhanced airway hyperresponsiveness associated with increased sputum eosinophilia [7] which are both observed during natural asthma exacerbation [9] or in response to natural exposure to pollen [8]. These results suggest low-dose allergen challenge may be a useful model to evaluate new therapies aimed at preventing airway responses to seasonal allergen exposure. Sputum induction has been shown to provide a noninvasive, reproducible measurement of airway eosinophils [10].

Glucocorticoids are highly effective for the treatment of asthma [11,12], improving airway hyperresponsiveness [13], some of the airway structural changes [14], and reducing inflammatory cells [15] and inflammatory mediators [16] in the airways of asthmatics. Regular treatment with inhaled budesonide is known to suppress bronchoconstrictor responses, airway hyperresponsiveness, and eosinophils in induced sputum following high dose allergen challenge [17]. However, the effects of regular treatment with inhaled corticosteroids on the airway responses to repeated low-dose allergen challenge has not been studied. There has also been recent acceptance for the use of low-dose inhaled budesonide as once daily therapy in the treatment of mild persisting asthma. Once again, however, low-dose once daily therapy has not been previously evaluated against allergen-induced airway responses. The rationale for this study therefore was to examine the effects of once daily budesonide treatment on allergen-induced airway hyperresponsiveness and airway inflammation following repeated low-dose allergen inhalation challenge to determine

if low, once daily doses of inhaled budesonide would attenuate allergen-induced airway responses and airway inflammation in an experimental setting designed to mimic regular allergen inhalation in atopic asthmatics.

Materials and methods

Subjects

Ten nonsmoking subjects (five males, five females) with mild allergic asthma were recruited for the study (Table 1). All subjects had mild asthma symptoms for more than 1 year, required only occasional use of inhaled β_2 -agonists, and had no asthma exacerbations or respiratory tract infections for at least 4 weeks before entering the study. Pollen-sensitive subjects were not tested during pollen seasons. Subjects were asked to withhold β_2 -agonists and caffeine-containing beverages for 8 h before visits to the laboratory. The protocol was approved by the Ethics Committee of McMaster University Health Science Center, and all subjects gave written informed consent to participate in the study. Only subjects who developed a late fall in FEV₁ of at least 15% on at least one occasion during the 3–7 h following high-dose allergen challenge were enrolled in the study. Two subjects (one male, one female) were removed from the study because of protocol violations. Thus, measurements from eight subjects were used for analysis.

Study design

A randomized, double-blind placebo-controlled cross-over study was designed to evaluate the effects of inhaled budesonide on repeated low-dose allergen-induced changes in airway physiology and airway inflammation (Fig. 1).

Table 1. Subject characteristics at screening visit

Sex	Age (dilution)	Allergen type (%pred)	Low-dose allergen (mg/mL)	FEV ₁	MCh PC ₂₀
M	21	HDM	1 : 32	92	1.64
F	21	Ragweed	1 : 512	85	8.89
F	28	HDM	1 : 64	97	12.23
M	29	Grass	1 : 8192	84	3.33
M	21	Ragweed	1 : 2048	103	7.2
M	49	Cat	1 : 64	94	2.83
M	37	HDM	1 : 1024	108	14.37
F	22	HDM	1 : 128	103	9.53
F	21	HDM	1 : 512	90	2.91
F	20	HDM	1 : 1024	91	0.93

FEV₁; forced expiratory volume in 1 second, MCh PC₂₀; provocative concentration of methacholine causing a 20% fall in FEV₁.

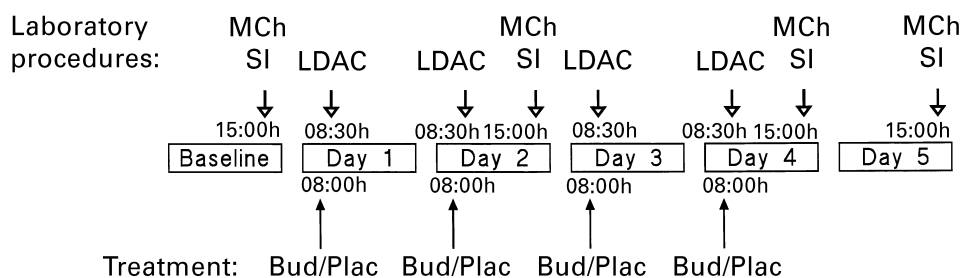


Fig. 1. Schematic of study design. MCh = methacholine PC₂₀, SI = sputum induction, LDAC = low-dose allergen challenge, Bud = budesonide treatment, Plac = placebo treatment.

Each subject completed a screening period consisting of a high-dose allergen challenge with a minimum of 2 weeks washout, followed by three treatment periods with the sequence of these treatments being computer-randomized. Four consecutive mornings at 8:00 am (day 1–day 4), subjects received either budesonide 400 µg, budesonide 100 µg, or placebo treatment administered by Turbuhaler® (Astra Draco, Lund, Sweden) 30 min before inhaling a low dose of allergen. This dose of allergen chosen was predicted based on the high-dose allergen challenge during the screening period, to result in a 5% early fall in the forced expiratory volume in 1 second (FEV₁). Measurements of FEV₁ were taken before and after low-dose allergen challenge to measure effects of treatment on the early response. The provocative concentration of methacholine causing a 20% fall in FEV₁ (methacholine PC₂₀) and sputum inflammatory cells were measured at baseline. On days 2, 4, and 5, the subjects reported to the laboratory at 3:00 p.m. for methacholine challenge and sputum induction (Fig. 1). Treatment periods were separated by at least a 1-week washout, as allergen-induced airway hyperresponsiveness and sputum eosinophilia has previously been shown to return to baseline within one week following repeated low dose allergen challenge [7]. If airway responsiveness had not returned to within one doubling concentration of the baseline methacholine PC₂₀ by the start of the next treatment period, the washout period was extended by 1 week.

High-dose allergen challenge

High-dose allergen challenge, as described by O'Byrne and colleagues [1] was performed during a screening period. Briefly, the allergen producing the largest skin weal diameter was diluted in 0.9% saline for inhalation. The concentration of allergen extract for inhalation was determined from a formula described by Cockcroft and coworkers [18] using the results from the skin test and the methacholine PC₂₀. The starting concentration of allergen extract for inhalation was two doubling concentrations below that predicted to cause a 15% early fall in FEV₁, and was inhaled

from a Wright nebulizer for 2 min. Allergen concentrations were doubled until a 15% fall in FEV₁ was observed 10 min after inhalation. Single measurements of FEV₁ were taken at 10 min, then again at 20, 30, 45, 60, 90 and 120 min following allergen inhalation, then each hour until 7 h after allergen inhalation. The early asthmatic response was taken to be the largest fall in FEV₁ within 2 h after allergen inhalation, and the late asthmatic response was taken to be the largest fall in FEV₁ between 3 h and 7 h after allergen inhalation. The concentration of allergen causing an early fall in FEV₁ of approximately 5% was used for all low-dose allergen challenges.

Low-dose allergen inhalation challenge

On the mornings of day 1 to day 4, subjects reported to the laboratory at 8:00 a.m. to inhale the study medication. After 30 min, subjects inhaled a predetermined concentration of allergen, administered as a single challenge, for 2 min from a Wright nebulizer as described by Sulakvelidze and colleagues [7]. FEV₁ was measured 10 min following inhalation and at 10 min intervals for 40 min.

Methacholine inhalation test

Methacholine inhalation challenge was performed as described by Cockcroft [19]. Subjects inhaled normal saline, then doubling concentrations of methacholine phosphate from a Wright nebulizer for 2 min. FEV₁ was measured at 30, 90, 180 and 300 s after each inhalation. Spirometry was measured with a Collins water-sealed spirometer and kymograph. The test was terminated when a fall in FEV₁ of 20% of the baseline value occurred, and the methacholine PC₂₀ was calculated.

Sputum analysis

Sputum was induced and processed using the method described by Pizzichini and coworkers [10]. Subjects inhaled 3%, 4% then 5% saline for 7 min each. The induction

was stopped when an adequate sample was obtained, or if the FEV₁ dropped 20% from baseline. Cell plugs were selected from the sample, separated from saliva, and weighed. Samples were mixed in four times their volume of 0.1% dithiothreitol (Sputolysin, Calbiochem Corp. San Diego, CA) and four times their volume of Dulbecco's phosphate buffered saline (DPBS) (Life Technologies Inc., Grand Island, NY, USA). The cell suspension was filtered through a 52- μ m nylon gauze (BNSH Thompson, Scarborough, Ontario, Canada) to remove debris, centrifuged at 426 g for 10 min, then supernatant was removed and stored at -70°C. The total cell count was determined using a Neubauer haemocytometer chamber (Hausser Scientific, Blue Bell, PA) and expressed as the number of cells per millilitre sputum. Cells were resuspended in DPBS at 0.75–1.0 $\times 10^6$ /mL and cytopspins were prepared on glass slides using 50 μ L of cell suspension and a Shandon III cyto-centrifuge (Shandon Southern Instruments, Sewickly, PA, USA), at 7 g for 5 min. Differential cell counts were obtained from the mean of two slides with 400 nonsquamous cells counted per slide stained with Diff Quik (American Scientific Products, McGaw Park, IL, USA). Metachromatic cell (mast cells and basophils) counts on slides stained with toluidine blue, were obtained from the mean of two slides with 1500 nonsquamous cells observed on each slide. Cytopspins were also prepared on aptex-coated slides for immunocytochemical staining, and fixed for 10 min in periodate-lysine-paraformaldehyde. Slides were stained using a murine monoclonal antibody to the activated form of human ECP, at 1.0 μ g/mL (EG2) (Kabi Pharmacia, Uppsala, Sweden). Briefly, slides were incubated overnight with the EG2 primary antibodies, and protein was detected the following day by the alkaline phosphatase antialkaline phosphatase (APAAP) method [20] using rabbit antimurine secondary antibodies, and monoclonal murine APAAP tertiary antibodies (DAKO, Denmark). All antibodies were diluted in 1.0% bovine serum albumin (Sigma Chemical Co, St. Louis, MO, USA) in wash buffer made up of DPBS, 0.01 M HEPES buffer (Life Technologies Inc., Grand Island, NY, USA) and 0.01% saponin (Sigma). Non-specific staining of the primary and secondary antibodies were blocked by incubation with 75% human AB serum and 25% normal rabbit serum (Sigma) diluted in wash buffer. Negative isotype controls (murine IgG₁, Sigma) for each slide were included using the same concentration as the primary antibody. The percentage of positive immunoreactive cells was determined from a count of 400 cells under light microscopy. Levels of ECP were measured in supernatant obtained from dithiothreitol-treated sputum samples, using a radio-immunoassay with a detection limit <2 ng/mL (Kabi Pharmacia, Uppsala, Sweden). The concentration of ECP in supernatant was multiplied by the dilution factor of nine, to be expressed as μ g ECP per millilitre sputum.

Statistics

Summary statistics are expressed as mean and SEM, with the exception of methacholine PC₂₀ measurements, which were made by linear interpolation of log dose response curves and expressed as geometric mean and geometric standard error of the mean (GSEM). Absolute values of repeated measurements were analysed for effects of allergen and budesonide using repeated measures ANOVA [21] with Newman-Keuls post-hoc test.

Results

The mean maximal early fall in FEV₁ on the 4 days after low-dose allergen challenge was $-9.2 \pm 1.1\%$. There was no attenuation of the early fall in FEV₁ with budesonide treatment, with the mean maximal early fall on the four challenge days being $-8.3 \pm 1.5\%$ with budesonide 100 μ g, and $-7.0 \pm 1.2\%$ with budesonide 400 μ g ($P = 0.16$). The early percentage fall in FEV₁ was significantly greater on day 2 compared to day 1 on both placebo and budesonide treatments ($P = 0.002$) (Fig. 2).

There was a significant allergen-induced reduction in the methacholine PC₂₀ following low-dose allergen challenge during placebo treatment, from 3.9 mg/mL (1.4 GSEM) at baseline to 2.2 mg/mL (1.7 GSEM) on day 4 ($P = 0.03$) (Fig. 3). Allergen-induced changes in methacholine PC₂₀ were no longer significantly different 24 h after the last challenge, being 2.7 mg/mL (1.8 GSEM) ($P = 0.58$). There was a significant protective effect of budesonide 400 μ g on allergen-induced methacholine PC₂₀, being 3.6 mg/mL (1.6 GSEM) on day 4 of the low-dose challenge, when compared to placebo ($P = 0.017$) (Fig. 3). The significant difference between placebo and budesonide 400 μ g persisted even the day following the repeated allergen inhalations. However, this attenuation of allergen-induced airway hyper-responsiveness did not occur during treatment with budesonide 100 μ g, the methacholine PC₂₀ being 1.43 mg/mL (1.42 GSEM) on day 4 of the low-dose challenge, which was significantly different from budesonide 400 μ g ($P = 0.018$) (Fig. 3).

The percentage sputum eosinophils measured during placebo treatment increased from $2.0 \pm 0.7\%$ at baseline, to $16.6 \pm 7.1\%$ after day 4 challenge ($P = 0.002$), and $12.2 \pm 4.3\%$ one day after the last challenge ($P = 0.08$) (Fig. 4). Budesonide treatment significantly attenuated the allergen-induced increase in percent sputum eosinophils after day 4 of the challenge when compared to placebo, to $5.6 \pm 1.8\%$ with budesonide 100 μ g ($P = 0.01$), and to $3.1 \pm 0.9\%$ with budesonide 400 μ g ($P = 0.004$) (Fig. 4). There was also an allergen-induced increase in the percent activated eosinophils (EG2-positive cells) from a baseline of $3.3 \pm 1.1\%$, to $22.8 \pm 7.9\%$ after day 4 of the low-dose

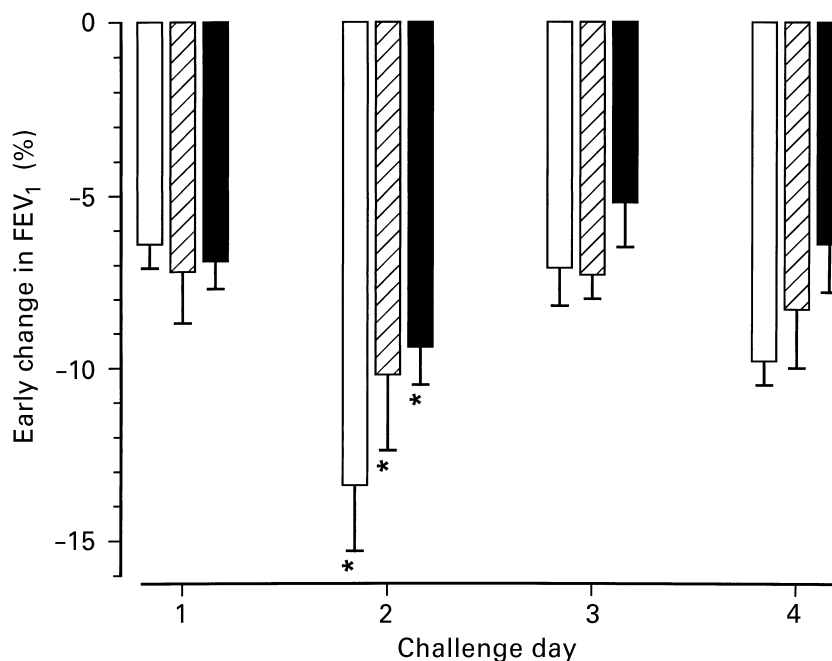


Fig. 2. Maximum percent fall in FEV₁ measured during the early asthmatic response on day 1 to day 4 following low-dose allergen inhalation challenges with placebo (open bars), budesonide 100 µg (hatched bars) and budesonide 400 µg (solid bars) pretreatment. * $P < 0.05$ compared to other challenge days.

allergen challenge ($P = 0.001$), and $15.7 \pm 6.6\%$ 24 h after the last challenge ($P = 0.09$). The allergen-induced increases in percent sputum EG2-positive cells after day 4 of the low dose allergen challenge were again significantly attenuated to $3.8 \pm 1.4\%$ with budesonide 100 µg ($P = 0.0009$) and to $4.1 \pm 1.6\%$ with budesonide 400 µg ($P = 0.0007$).

The percent metachromatic cells increased from

$0.02 \pm 0.01\%$ at baseline, to $0.18 \pm 0.07\%$ after day 2 challenge, $0.1 \pm 0.02\%$ after day 4 challenge, and $0.23 \pm 0.09\%$ 24 h after the last challenge, however, this was not statistically significant ($P = 0.06$) (Fig. 4). There was no significant effect of budesonide 100 µg or 400 µg on allergen-induced percent metachromatic cells ($P = 0.13$).

The percentage sputum neutrophils measured during placebo treatment was $47.1 \pm 10.9\%$ at baseline,

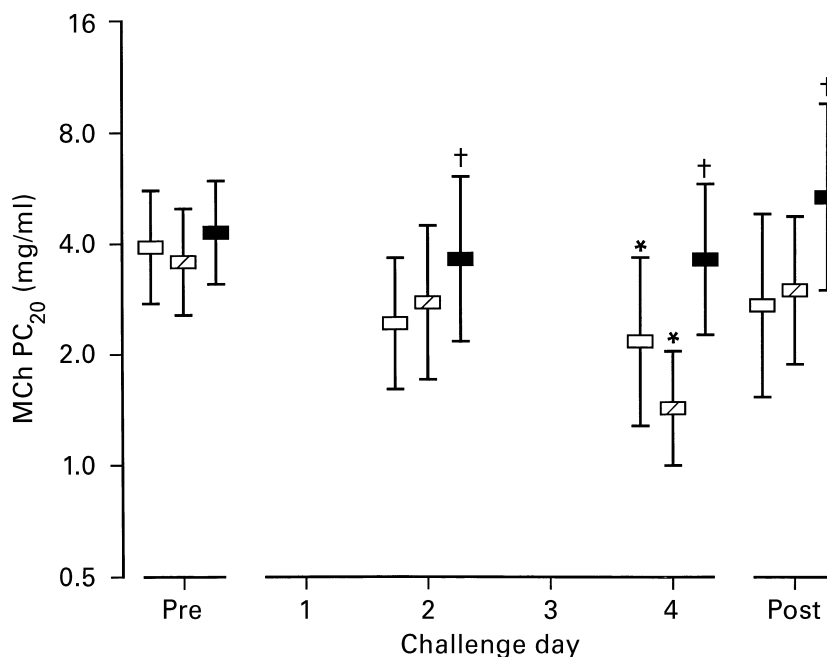


Fig. 3. Methacholine PC₂₀ measured before, 7 h after day 2 and day 4 low-dose allergen inhalation challenges, and 1 day following the last challenge with placebo (open rectangles), budesonide 100 µg (hatched rectangles), and budesonide 400 µg (solid rectangles) pretreatment. * $P < 0.05$ difference from baseline, [†] $P < 0.05$ difference between placebo and budesonide.

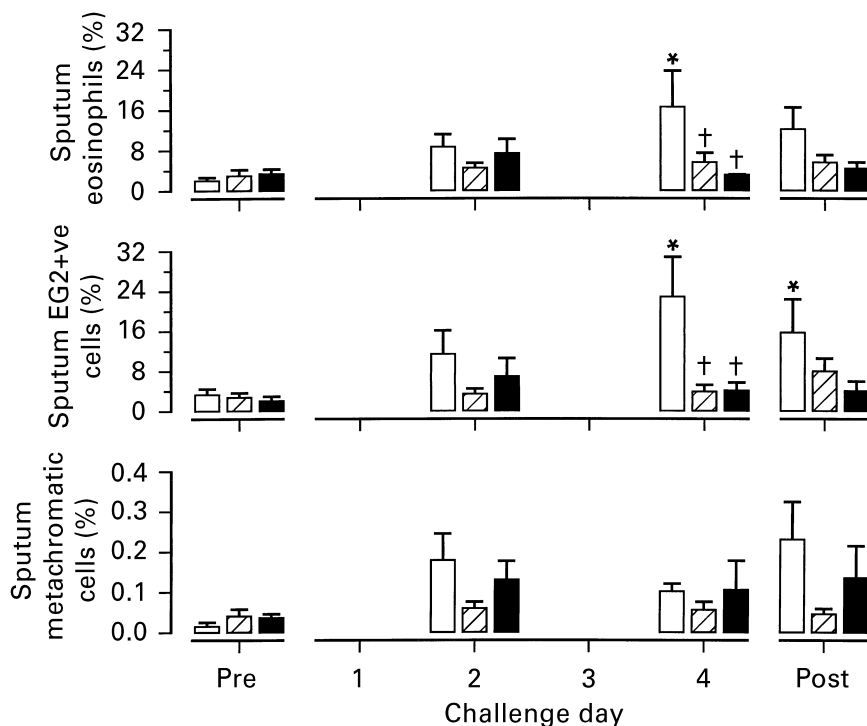


Fig. 4. Percent sputum eosinophils (top panel), activated eosinophils (middle panel) and metachromatic cells (bottom panel) measured before, 7 h after day 2 and day 4 low-dose allergen inhalation challenges, and 1 day following the last challenge with placebo (open bars), budesonide 100 µg (hatched bars), and budesonide 400 µg (solid bars) pretreatment. * $P < 0.05$ difference from baseline, † $P < 0.05$ difference between placebo and budesonide.

42.1 ± 9.4% after day 2 challenge, 41.1 ± 8.2% after day 4 challenge, and 45.5 ± 7.2% one day after the last challenge, with no effect of allergen or budesonide treatment ($P > 0.05$). The percentage sputum macrophages measured during placebo treatment was 47.5 ± 9.6% at baseline, 45.8 ± 8.8% after day 2 challenge, 41.3 ± 7.6% after day 4 challenge, and 42.5 ± 8.0% one day after the last challenge, with no effect of allergen or budesonide treatment ($P > 0.05$).

The level of ECP measured from sputum fluid phase during placebo treatment increased significantly from 707 ± 309 µg/L at baseline, to 2078 ± 813 µg/L after day 4 of the low-dose allergen challenge ($P = 0.03$), and 1368 ± 619 µg/L 24 h after the last challenge ($P = 0.08$). With budesonide 100 µg the level of ECP changed from 420 ± 130 µg/L at baseline, to 815 ± 254 µg/L after day 4 challenge, and 1200 ± 193 µg/L 24 h after the last challenge. With budesonide 400 µg, the level of ECP changed from 478 ± 148 µg/L at baseline, to 595 ± 193 µg/L after day 4 challenge, and 462 ± 88 µg/L 24 h after the last challenge. However, the effect of budesonide was not statistically different when compared to placebo ($P = 0.08$).

Discussion

This study has demonstrated that allergen-induced airway hyperresponsiveness and airway eosinophilia which develops over 4 days of repeated low-dose allergen inhalation challenge, were significantly attenuated by treatment with 400 µg budesonide inhaled once daily, 30 min prior to

each allergen challenge. A lower dose of budesonide, 100 µg once daily, significantly attenuated the allergen-induced sputum eosinophilia, but not the allergen-induced airway hyperresponsiveness. These observations using a repeated low-dose allergen challenge are novel, and extend previous observations that regular treatment with 200 µg budesonide inhaled twice daily reduces high dose allergen-induced airway hyperresponsiveness and airway eosinophilia [17].

The method of repeated low-dose allergen challenge has been developed to mimic the natural course of allergic asthma more closely than the conventional single high-dose allergen inhalation challenge. Indeed, similar increases in airway hyperresponsiveness and airway inflammation have been described by several other groups, who have studied these indices during natural pollen exposure during a pollen season [8,22–25]. However, the use of repeated low-dose challenge does not fully address the criticism that high-dose allergen challenge involves a brief single exposure to a high dose of allergen, which would not often occur in the majority of asthmatics. During repeated low-dose allergen challenge, the allergen is also inhaled only once throughout the day, while natural allergen exposure is likely to be of even lower dose, and more prolonged. This method does, however, allow the development of eosinophilic airway inflammation and airway hyperresponsiveness with very mild degrees of bronchoconstriction.

Inhaled corticosteroid treatment given once daily is effective in initiating and maintaining clinical control of

asthma [26], and twice daily reduces the immediate airway responsiveness to high-dose allergen challenge as measured by allergen PC₁₅ [27]. In addition, single-dose inhaled corticosteroid treatment prior to high-dose allergen inhalation is effective in attenuating the late airway response [28,29]. That we have been able to demonstrate significant attenuation of allergen-induced airway responses in a group of eight subjects, suggests the repeated low-dose allergen challenge might be a useful, more clinically relevant model for screening the efficacy of potential anti-inflammatory drugs in allergic asthmatics.

Repeated low-dose allergen challenge resulted in the development of airway hyperresponsiveness and sputum eosinophilia coincidentally after 4 days of challenge, supporting previous studies indicating the relationship between airway eosinophils and airway hyperresponsiveness [3,7,17]. That we did not observe a significant effect of budesonide on airway levels of ECP may be due to the high baseline values measured with placebo treatment as well as the large between-subject variability. There have been several mechanisms described to explain a role for eosinophils in the development of airway hyperresponsiveness. Eosinophils are known to release several basic proteins, which are toxic to the airways. For example, major basic protein, can mediate airway hyperresponsiveness by inhibiting neuronal M₂ muscarinic receptor function [30,31]. In addition, major basic protein and eosinophil cationic protein have been shown to damage airway epithelium [32,33]. The previously described attenuation of both allergen-induced airway eosinophilia and airway hyperresponsiveness with inhaled corticosteroids [17] supported that this association was causal. Suppression of allergen-induced airway sputum eosinophilia by budesonide 100 µg without suppression of airway hyperresponsiveness was an unexpected finding. Dissociation between allergen-induced airway responses and airway eosinophil levels, however, has also been reported following pharmacological treatment with an anti-IL-5 antibody [34]. Lack of a correlation between airway hyperresponsiveness and eosinophils measured in bronchoalveolar lavage fluid, sputum and bronchial biopsy samples confirms this dissociation is not dependent on the sampling site [35]. Together, these studies suggest that the presence of airway eosinophils alone may not be necessary for the development of allergen-induced airway hyperresponsiveness. The mechanisms for this are not known. However, mediator release by allergen-activated mast cells may contribute to the allergen-induced airway hyperresponsiveness. Mast cells and basophils activated by specific allergen may not be sensitive to corticosteroids, as we have shown no effect of budesonide on allergen-induced increases in sputum metachromatic cells in the present study, or in studies using high dose allergen challenge [17]. Furthermore, corticosteroid is unable to inhibit

mediator release from mast cells in the context of neurogenic inflammation [36], a phenomenon likely to occur in the airway following allergen exposure [37]. In addition, inhaled corticosteroids attenuate but do not abolish allergen-induced early responses following high-dose allergen challenge [17], which are known to be mast cell-mediated events. In this repeated low-dose allergen model, the inability of the lower dose of budesonide to prevent the release of preformed mediators from mast cells following allergen challenge may permit initiation of the inflammatory cascade leading to airway hyperresponsiveness. The higher dose of budesonide therefore appears to be necessary to inhibit the development of allergen-induced airway hyperresponsiveness. Extending the duration of pretreatment with budesonide 100 µg before low-dose allergen challenge may abolish the allergen-induced airway hyperresponsiveness, as this has been observed after high-dose allergen challenge with 1 week pretreatment with budesonide 200 µg BID [17].

Another surprising result in this study was that the airway response to low-dose allergen was significantly greater during the day 2 challenge when compared to the days 1, 3 and 4 challenges, demonstrating a transient airway hyperresponsiveness to allergen. The early airway response to challenges on days 3 and 4 were similar to that of day 1, which reflects trends observed by Sulakvelidze and coworkers [7]. By contrast, increases in methacholine airway responsiveness did not develop until day 4 of challenge, suggesting that different mechanisms, such as mast cell activation, are involved in airway hyperresponsiveness to specific and nonspecific stimuli. Whether or not activation of mast cells contributes to the enhanced allergen-induced early response observed on day 2 is not known, however, employing AMP inhalation challenge as a measure of mast cell activation would help to clarify the role of the mast cell in airway responsiveness to inhaled allergen.

This study has demonstrated, that once daily dosing of inhaled budesonide 400 µg is effective in preventing allergen-induced airway responses. Several studies have demonstrated the clinical efficacy of once daily inhaled budesonide in patients with mild to moderate persisting asthma [26,38], and furthermore, in the control of nasal symptoms associated with perennial [39] and allergen-induced nasal rhinitis [40]. The results of the present study raise the possibility that once daily treatment with low doses of budesonide may be beneficial to atopic asthmatics during seasonal allergen exposure. This issue, however, will need to be evaluated in an appropriately designed study during seasonal allergen exposure.

In conclusion, this study has confirmed our previous observations that repeated low-dose allergen challenge causes an increase in sputum eosinophils, activated eosinophils and ECP, as well as increasing methacholine airway

responsiveness. These inflammatory changes were resolving by 24 h after the last allergen challenge. The allergen-induced changes in airway hyperresponsiveness and airway eosinophils were attenuated by treatment during the repeated low-dose challenge by once daily, low doses of 400 µg inhaled budesonide. A once daily lower dose of 100 µg was effective in preventing the allergen-induced airway eosinophils, but not hyperresponsiveness.

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