

Original article

Single dose topical corticosteroid inhibits IL-5 and IL-13 in nasal lavage following grass pollen challenge

Background: Nasal lavage is a noninvasive method of obtaining inflammatory exudates following nasal allergen challenge (NAC), and permits cells and released mediators to be evaluated.

Objective: To determine the effects of a single dose of topical steroid on eosinophils and levels of chemokines and cytokines in nasal lavage fluid following NAC in patients with allergic rhinitis.

Methods: Patients with grass pollen seasonal allergic rhinitis ($n = 32$) out of the allergy season received either nasal budesonide (100 µg per nostril) or matched placebo before allergen challenge in a double blind two-way crossover design. A semi-automated mixed bead array system was employed to measure multiple chemokines and cytokines in small volumes (50 µl) of nasal lavage supernatants.

Results: Following NAC there was a rapid onset of nasal symptoms together with nasal eosinophilia, and the appearance of IL-5 and IL-13 in lavages between 4 and 8 h. Elevated levels of eotaxin, RANTES, IL-8 and MCP-1 were also detected following allergen challenge. A single dose of nasal budesonide caused a decrease in symptoms ($P < 0.05$) and nasal eosinophils ($P < 0.05$) with selective abrogation of IL-5 and IL-13 responses ($P < 0.05$), but a lack of effect on levels of eotaxin, RANTES, IL-8 and MCP-1.

Conclusion: This study suggests that a single dose of nasal steroid has the capacity to selectively abolish IL-5 and IL-13 responses following NAC. This model should be convenient for testing novel anti-inflammatory and immunoregulatory agents intended for the treatment of allergic rhinitis.

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Key words: allergen challenge, budesonide, eosinophils, IL-13, IL-5, Nasal lavage.

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Accepted for publication 18 May 2005

The inflammatory response found in allergic rhinitis is characterized by the presence of T helper 2 lymphocytes (T_H2 cells), IgE-dependent mast cell degranulation, and increased numbers of eosinophils (1). Increased activity of specific T_H2 cells contribute to allergic inflammation, coordinating the activity of cells including mast cells and eosinophils (2). The T_H2 cells produce the important immunoregulatory cytokines IL-13 and IL-5 (3) and increased levels of nasal mRNA of these cytokines have been found following allergen challenge (4, 5).

The nasal inflammatory exudates can be measured in nasal lavage fluid or fluid adsorbed on filter paper strips placed on the nasal mucosa. Following allergen challenge increases in IL-5 and eotaxin have been detected in nasal lavage fluid (6, 7). In addition, postallergen challenge

increases in granulocyte macrophage-colony stimulating factor (GM-CSF), IL-5, IL-8, MIP-1α, and RANTES (8–10) have been detected using filter paper strips.

Topical corticosteroids are recognized as first line therapy for more severe and persistent forms of allergic rhinitis by the Allergic Rhinitis and its Impact on Asthma (ARIA) global guidelines (11). A single dose of topical corticosteroid has been shown to reduce levels of GM-CSF and IL-5 detected by absorption with filter paper following nasal challenge with grass pollen in allergic rhinitis (4, 9). Maintenance treatment with topical steroids exerts a range of anti-inflammatory nasal effects, and following nasal allergen challenge (NAC) has been shown to inhibit levels of cytokines such as IL-1β, GM-CSF (10) and TNF-α (12), as well as chemokines such as eotaxin (7), RANTES, MIP-1α, and IL-8 (10). Monoclonal anti-IgE antibody has recently been shown to inhibit NAC responses (13).

In the present study, we have assessed the nasal mucosal output of eosinophils and a range of chemokines

Abbreviations: NAC, nasal allergen challenge; T_H2, T helper type 2 lymphocytes; PBS, phosphate-buffered saline.

and cytokines in nasal lavage at a series of time points after allergen challenge. We studied patients with seasonal allergic rhinitis out of the grass pollen season. We have utilized a highly sensitive bead array immunoassay to assess cytokine and chemokine profiles, and studied the effects of a single dose of topically applied corticosteroid.

Methods

Patients

Thirty-two patients with grass pollen seasonal allergic rhinitis were studied. All subjects were nonsmokers (19–53 years) (demographic data is shown in Table 1). A history of summer grass pollen allergic rhinitis for at least 2 years was confirmed by a positive skin prick test to Timothy grass pollen extract (Soluprick, *phleum pratense*; ALK, Horsholm, Denmark). i.e. ≥ 3 mm weal compared with the negative control. To avoid natural allergen provocation, all subjects were studied outside the grass-pollen season. Patients were excluded if they had received topical or oral antiallergy medication in the previous 6 months or immunotherapy in the previous 5 years and evidence of active rhinitis on examination of the nasal mucosa at study entry. The study was approved by the local Research Ethics Committee, and informed written consent was obtained from each subject.

Study design

Patients were randomized to receive either a single dose of nasal budesonide 200 μg (100 μg in each nostril) or matched placebo in a double-blind two-way crossover design, 1 week apart. Local NAC with Timothy grass pollen (Aquagen, *phleum pratense*; ALK) followed 30 min after treatment via a nasal pump spray delivery device (Bidose; Valois S.A., Marly le Roi, France). Nasal lavage was performed at 0, 1, 2, 4, 6, 8 and 24 h after allergen challenge.

Nasal allergen challenge procedure

Nasal allergen challenge was administered in the morning of each visit by means of a nasal pump spray (Bidose) that delivered 100 μl into each nostril. The NAC with Timothy grass pollen freeze-dried extract was given as a total nose dose of 1000 biological units (500BU per actuation to each nostril). A dose of 320BU has previously been shown to provoke immediate and late responses (14).

Table 1. Subject characteristics

Characteristic	
Age (years)	
Mean	29.5
Range	19–53
Sex (F/M)	10/22
Race	
White	23
Black	5
Asian	3
Oriental	1
Total serum IgE (IU/ml)	
Mean	271.1
SD	419.1
Baseline blood eosinophils ($\times 10^9$ cells/l)	
Mean	0.20
SD	0.12

Nasal lavage procedure and processing

A modified nasal pool technique was adapted from that used in previous studies by Greiff et al. (15). Nasal lavage with Dulbecco's phosphate-buffered saline (PBS) was performed using a 10 ml syringe attached by tubing to a nasal adapter or olive, rinsing with 6 ml of prewarmed PBS (37°C). The subjects were seated in a forward-flexed neck position (60° from the upright position) to prevent fluid from reaching the nasopharynx. To ensure adequate washing, the lavage fluid (6 ml) was passed slowly into the nasal cavity and then left to dwell for 30 s. The fluid was withdrawn into the syringe and flushed back into the nasal cavity 30 times in 2 min. Care was taken to maintain a tight seal between the olive and nostril throughout. The nasal lavage samples were initially centrifuged at 4°C for 10 min at 400 *g* to generate a supernatant which was collected for later fluid phase analysis. The cell pellet was resuspended in 0.01%, dithiothreitol in PBS. The samples were gently agitated on a rolling mixer for 10 min and then centrifuged again to generate a supernatant. The supernatant was discarded and the remaining pellet resuspended in PBS. An initial estimate of total cell count was made using an improved Neubauer haemocytometer (Glaswarenfabrik, Sondheim, Germany). The cell suspension was diluted and cytospin slides were produced. Slides were stained with Diff-Quik stain and differential cell counts were determined by assessment of 400 leucocytes on the cytospin.

Measurement of mediators

The nasal lavage supernatants were centrifuged through 0.22 μm cellulose acetate filters (Costar spin-X®) at 4°C for 30 min at 13 000 *g* to remove residual mucous before analysis. The levels of human eotaxin, RANTES, IL-5, IL-8, IL-13 and MCP-1 were analysed using a multiplexed bead array system (Upstate Ltd, Milton Keynes, UK) in a blind manner. Samples were read using a commercially available Luminex100™ IS instrument (Luminex Corporation, Austin, TX, USA). This chemokine assay is designed for analysis of multiple quantitative measurements of supernatants. Using this 96-well microtitre plate-formatted assay it is possible to profile the level of multiple chemokines in a single well using as little as 50 μl of sample. Detailed manufacturer's protocol instructions were followed. However, to increase sensitivity the gain of the RP1 reporter channel was increased from a value of 3885 to 9000 and a final late wash step was added to reduce the associated increase in background. The sensitivities thus obtained for each cytokine and chemokine were 1.5 pg/ml for IL-5, IL-8, IL-10, IL-13, MCP-1, and Eotaxin and 2.3 pg/ml for RANTES.

Bradford protein assay

The Bradford protein assay (16) was performed in 96-well plates. Briefly, 10 μl of standard (BSA standards in PBS from 2 to 0.03 mg/ml) or sample per well and mixed with 200 μl of Bradford reagent [100 mg of Coomassie Brilliant Blue G (colour index 42655) in 50 ml of 95% ethanol, to which was added 100 ml of concentrated 85% phosphoric acid followed by 1 l of water]. After 5 min the plates were read at 620 nm.

Nasal symptoms

Nasal symptoms were recorded before and 1, 2, 4, 6, 8 and 24 h after NAC. Nasal congestion, rhinorrhea, sneezing and nasal itch, were scored from 0 to 3 (0 = no, 1 = mild, 2 = moderate, 3 = severe symptoms).

Statistical analyses

The significance of the change from baseline and the difference between active and placebo was tested at each time point, with the nonparametric Wilcoxon signed rank test. Correlations were evaluated by the Spearman rank correlation test. A P -value of <0.05 was considered significant.

Results

Effects of NAC in placebo group

Following allergen challenge, nasal symptoms, eosinophils and cytokines levels changed significantly (Figs 1 and 2). Postallergen challenge nasal symptoms were almost immediate ($P < 0.01$ at 1 and 2 h) and lasted for up to 2 h ($P < 0.01$) before gradually abating. The number of eosinophils in nasal lavage fluid was raised at 1–6 h, and then gradually decreased, but remained elevated at 24 h (Fig. 1). During the late observation hours, the number of eosinophils gradually increased to a maximum at 6 h

($P < 0.01$), which persisted until 8 h ($P < 0.01$) and returned towards baseline again at 24 h ($P > 0.05$). Similarly, the level of eotaxin increased in the early and late phases (but not significantly). Levels of IL-5 ($P < 0.01$ from 6 to 24 h), IL-13 ($P < 0.01$ from 4 to 24 h), RANTES ($P < 0.05$ at 2 h), MCP-1 ($P < 0.05$ at 2 and 8 h) and IL-8 (not significant) measured in nasal lavage fluid postallergen challenge were increased. The lavage levels of eosinophil counts and IL-5 showed a strong statistical correlation at 6 h ($r = 0.53$, $P = 0.01$) and 8 h ($r = 0.42$, $P = 0.03$). No other significant correlations were seen between eosinophils and chemokines or cytokines.

Effects of nasal budesonide

A single dose of nasal budesonide had a range of effects on nasal responses. Following NAC there was a statistically significant decrease in symptoms at 8 and 24 h ($P < 0.05$) and of eosinophils in nasal lavage at 4–8 h

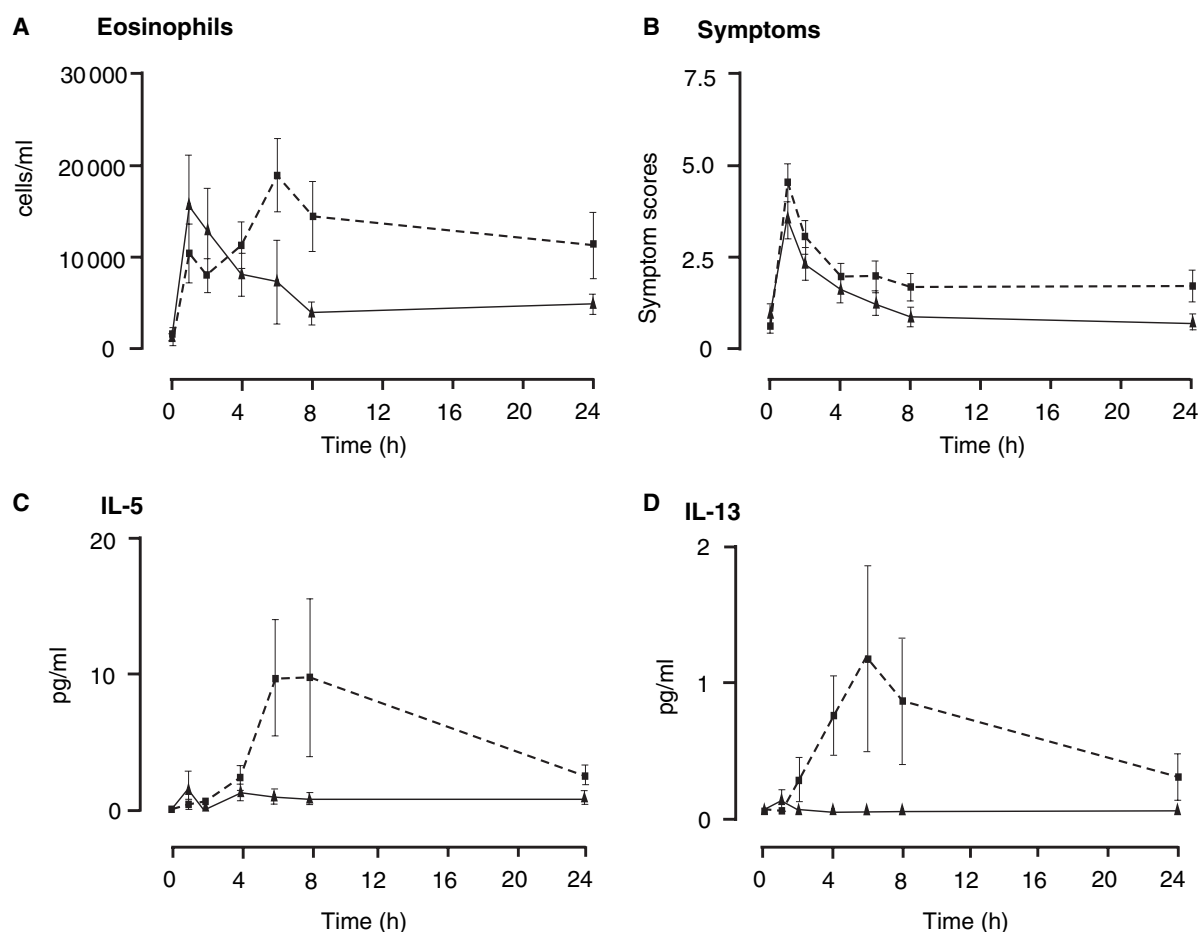


Figure 1. Eosinophil cell counts (A), symptom scores (B), IL-5 levels (C) and IL-13 levels (D) before and at various time points following allergen challenge either after placebo (dotted line) or single dose budesonide (solid line) pretreatment. Values presented are mean $\pm$ SEM ($n = 32$).

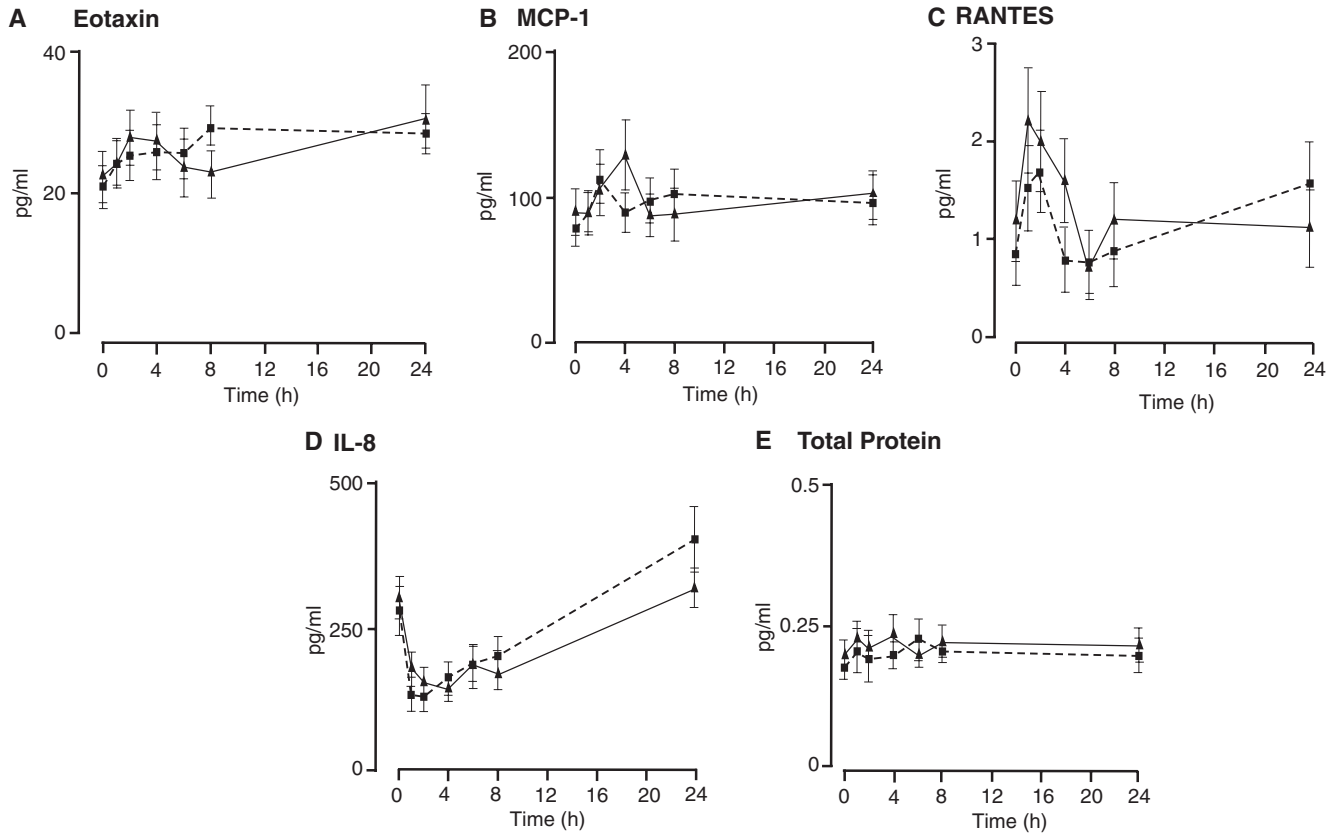


Figure 2. Eotaxin (A), MCP-1 (B), RANTES (C) and IL-8 levels (D) before and at various time points following allergen challenge either after placebo (dotted line) or single dose budesonide (solid line) pretreatment. Values presented are mean $\pm$ SEM ($n = 32$).

($P < 0.05$). IL-13 was significantly decreased at 2, 4, 6, 8 and 24 h (at each time point $P < 0.05$). IL-5 was significantly decreased at 6, 8 and 24 h ($P < 0.05$) (Fig. 1). There was no statistically significant inhibition in total protein levels or the levels of eotaxin, MCP-1, RANTES, or IL-8 (Fig. 2).

Following lavage with 6 ml of fluid a mean recovery of 5.07 ml ($\pm$ SD 0.44) was obtained when considering all subjects at baseline. There was no significant difference in the recovered volumes of nasal lavage fluid between the nasal budesonide and placebo limbs in this crossover study. The interindividual difference has been analysed in terms of volume of recovery and has been shown to have a coefficient of variation of 8.9%. As a marker of dilution, total protein levels were measured in nasal lavage fluid and there was no significant difference between budesonide and placebo treatment arms (Fig. 2).

As the recovery rate of nasal lavage fluids could vary from subject to subject and from lavage to lavage, levels of chemokines and cytokines were also analysed after normalization by concentration of total protein. The results obtained did not differ significantly from those obtained when the levels of chemokines and cytokines were expressed as a concentration in nasal lavage fluids (data not shown). For this reason, all lavage data are

presented as absolute concentrations and were not normalized to any other parameters.

Discussion

The principle finding of our study was the ablation of IL-5 and IL-13 responses following allergen challenge in response to a single dose of nasal steroid. This suggests an important role for these cytokines in the pathogenesis of allergic disease and demonstrates an important therapeutic mechanism of action of steroids. These data are of interest in part because of the potential importance of IL-5 and IL-13 in the pathogenesis of allergic airways disease and the current lack of experimental clinical models that can give direct technically robust information on the therapeutic potency of airway steroids. This study provides further evidence that nasal provocation can be used in phase II, proof of concept, clinical studies as part of a clinical drug development plan.

Within 2 h of a single dose of topical budesonide there was inhibition of allergen challenge induced mucosal output of IL-5 and IL-13 and within 6 h there is an inhibition of eosinophil influx and within 8 h there is an inhibition in symptoms. Thus, this study presents new

evidence that budesonide exerts anti-inflammatory airways effects with a rapid onset of action. The present data also demonstrates that single dose pretreatment with budesonide is sufficient for inhibition of nasal late phase cytokine generation.

Similarly, studies of asthmatic bronchi support the hypothesis that effects obtained a few hours after topical airway application of steroids are therapeutically relevant. Single dose inhaled steroid inhibits allergen challenge induced late phase exudation and obstruction (17). A single dose of inhaled fluticasone inhibits adenosine 5'-monophosphate (AMP)-induced bronchoconstriction (18, 19). In chronic asthma, budesonide significantly increases peak expiratory rate during the first 8 h after drug inhalation (20), and a single dose of inhaled budesonide causes decreased induced sputum eosinophils and airway hyperresponsiveness 6 h after dosing (21).

The traditional genomic theory of steroid action, involving direct interaction with DNA or involving cross-talk with other transcription factors, does not fully explain the rapid effects of steroids. It is thought that these rapid nongenomic actions are mediated by a family of membrane receptors (22, 23). Membrane receptors are linked to a number of intracellular signalling pathways acting through G-protein coupled receptors and kinase pathways. Of particular importance may be changes in bronchial blood flow induced by inhaled corticosteroids (24). In addition, the classical receptor is associated with a number of kinases and phosphatases within the inactive GR/hsp90 complex (25), and these enzymes are released upon hormone binding (26).

Theoretically, the present reduction of cytokine recovery from the nasal mucosa may in part, be secondary to local vasoconstrictor effects causing reduced vascular permeability (27). We have shown a selective reduction in IL-5 and IL-13 with no inhibition of total protein levels or inhibition of a range of other cytokines and chemokines. This would suggest the mechanism of inhibition of both IL-5 and IL-13 and eosinophil influx is not entirely due to nonselective vascular permeability effects.

The ablation of IL-5 and IL-13 responses by budesonide may be explained by action on the transcription factor GATA-3 (28), which has recently been described in

studies highlighting its critical role in T_H2 cell differentiation and allergic airway inflammation (29). GATA-3 may be the principle chromatin-remodelling factor around the IL-4/IL-5/IL-13 locus in cells stimulated with antigen and IL-4 (30–32).

The pretreatment with a single dose of budesonide had no effect on the nasal symptoms observed during immediate nasal response to allergen challenge. This is in agreement with a study that showed lack of effect on nasal symptoms after 2-h pretreatment with a single dose of steroid in a NAC model (33). Importantly, however, this study demonstrates a statistically significant decrease in symptoms at the later time points of 8 and 24 h.

Topical allergen challenge can be carried out with a single allergen challenge administration using nasal spray, filter paper, or lavage delivery devices (14). It can also be carried out with multiple repeat allergen challenges to titrate a given symptom score (33), or with controlled allergen exposure in a challenge chamber unit (34). In this study we have used a specialized nasal delivery device (Bidose nasal applicator; Valois) which can accurately deliver 100 µl of challenge agent, in this case Timothy grass pollen, precisely to each nostril.

In summary, the nasal allergy model has many attractive features for clinical pharmacology assessment of novel therapeutics. This is with the proviso that responses in the upper airway may not mirror those in the lower airways. Nasal lavage is a simple and noninvasive technique which can be used at frequent intervals before and after allergen challenge to describe responses at the nasal mucosal surface in an easy to recruit patient group. This present experimental model may be used to explore both cytokine involvement in allergic airway inflammation and potency as well as onset of action of airway glucocorticoid compounds.

Acknowledgments

We would like to thank Mrs Jackie Turner for her expert statistical advice. This work was supported by funding from R.W. Johnson Pharmaceutical Research.

References

1. Gelfand EW. Inflammatory mediators in allergic rhinitis. *J Allergy Clin Immunol* 2004;**114**:S135–S138.
2. Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;**111**:450–463.
3. Hershey GK. IL-13 receptors and signalling pathways: an evolving web. *J Allergy Clin Immunol* 2003;**111**:677–690.
4. Masuyama K, Till SJ, Jacobson MR, Kamil A, Cameron L, Juliusson S et al. Nasal eosinophilia and IL-5 mRNA expression in seasonal allergic rhinitis induced by natural allergen exposure: effects of topical corticosteroids. *J Allergy Clin Immunol* 1998;**102**:610–617.

5. Ghaffar O, Laberge S, Jacobson M, Lowhagen O, Rak S, Durham S et al. IL-13 mRNA and immunoreactivity in allergen-induced rhinitis: comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997;**17**:17–24.
6. Terada N, Hamano N, Kim WJ, Hirai K, Nakajima T, Yamada H et al. The kinetics of allergen-induced eotaxin level in nasal lavage fluid: its key role in eosinophil recruitment in nasal mucosa. *Am J Respir Crit Care Med* 2001;**164**:575–579.
7. Greiff L, Petersen H, Mattsson E, Andersson M, Erjefalt JS, Linden M et al. Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. *Clin Exp Allergy* 2001;**31**:1321–1327.
8. Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994;**149**:339–344.
9. Linden M, Svensson C, Andersson E, Andersson M, Greiff L, Persson CG. Immediate effect of topical budesonide on allergen challenge-induced nasal mucosal fluid levels of granulocyte-macrophage colony-stimulating factor and interleukin-5. *Am J Respir Crit Care Med* 2000;**162**:1705–1708.
10. Sim TC, Reece LM, Hilsmeier KA, Grant JA, Alam R. Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995;**152**:927–933.
11. Bousquet J, Van Cauwenberge P, Khaltaev N. and the WHO Panel. Allergic rhinitis and its impact on asthma. ARIA. In collaboration with the World Health Organization. *J Allergy Clin Immunol* 2001;**108**:S1–S315.
12. Ciprandi G, Tosca MA, Passalacqua G, Canonica GW. Intranasal mometasone furoate reduces late-phase inflammation after allergen challenge. *Ann Allergy Asthma Immunol* 2001;**86**:433–438.
13. Hanf G, Noga O, O'Connor A, Kunkel G. Omalizumab inhibits allergen challenge-induced nasal response. *Eur Respir J* 2004;**23**:414–418.
14. Varney VA, Jacobson MR, Sudderick RM, Robinson DS, Irani AM, Schwartz LB et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. Identification of activated T lymphocytes, eosinophils, and neutrophils. *Am Rev Respir Dis* 1992;**146**:170–176.
15. Greiff L, Pipkorn U, Alkner U, Persson CGA. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990;**20**:253–259.
16. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;**72**:248–254.
17. Parameswaran K, Inman MD, Watson RM, Morris MM, Efthimiadis A, Ventresca PG et al. Protective effects of fluticasone on allergen-induced airway responses and sputum inflammatory markers. *Can Respir J* 2000;**7**:313–319.
18. Ketchell RI, Jensen MW, Lumley P, Wright AM, Allenby MI, O'Connor BJ. Rapid effect of inhaled fluticasone propionate on airway responsiveness to adenosine 5'-monophosphate in mild asthma. *J Allergy Clin Immunol* 2002;**110**:603–606.
19. Luijk B, Kempersford RD, Wright AM, Zanen P, Lammers JW. Duration of effect of single-dose inhaled fluticasone propionate on AMP-induced bronchoconstriction. *Eur Respir J* 2004;**23**:559–564.
20. Dahl R, Johansson S. Importance of duration of treatment with inhaled budesonide on the immediate and late bronchial reaction. *Eur J Resp Disease* 1982;**63**(Suppl. 122):167–175.
21. Gibson PG, Saltos N, Fakes K. Acute anti-inflammatory effects of inhaled budesonide in asthma: a randomized controlled trial. *Am J Respir Crit Care Med* 2001;**163**:32–36.
22. Norman AW, Mizwicki MT, Norman DPG. Steroid-hormone rapid actions, membrane receptors and a conformational ensemble model. *Nature Reviews* 2004;**3**:27–41.
23. Chen Y.-Z, Qiu J. Pleiotropic signaling pathways in rapid, nongenomic action of glucocorticoid. *Mol Cell Biol Res Commun* 1999;**2**:145–149.
24. Mendes ES, Pereira A, Danta I, Duncan RC, Wanner A. Comparative bronchial vasoconstrictive efficacy of inhaled glucocorticosteroids. *Eur Respir J* 2003;**21**:989–993.
25. Adcock IM, Maneechotesuwan K, Usmani O. Molecular interactions between glucocorticoids and long-acting beta2-agonists. *J Allergy Clin Immunol* 2002;**110**:S261–S268.
26. Croxtall JD, van Hal PT, Choudhury Q, Gilroy DW, Flower RJ. Different glucocorticoids vary in their genomic and non-genomic mechanism of action in A549 cells. *Br J Pharmacol* 2002;**135**:511–519.
27. Kumar SD, Brieve JL, Danta I, Wanner A. Transient effect of inhaled fluticasone on airway mucosal blood flow in subjects with and without asthma. *Am J Respir Crit Care Med* 2000;**161**:918–921.
28. Rofojo D, Liberman A, Giacomini D, Nagashima A, Graciarena M, Echenique C et al. Integrating systemic information at the molecular level: cross talk between steroid receptors and cytokine signaling on different target cells. *Ann NY Acad Sci* 2003;**992**:196–204.
29. Nakamura Y, Christodoulouopoulos P, Cameron L, Wright E, Lavigne F, Toda M et al. Upregulation of the transcription factor GATA-3 in upper airway mucosa after in vivo and in vitro allergen challenge. *J Allergy Clin Immunol* 2000;**105**:1146–1152.
30. Ouyang W, Lohning M, Gao Z. Stat6-independent GATA-3 autoactivation directs IL-4-independent Th2 development and commitment. *Immunity* 2000;**12**:27–37.
31. Siegel MD, Zhang DH, Ray P, Ray A. Activation of the interleukin-5 promoter by CAMP in murine EL-4 cells requires the GATA-3 and C/EBP elements. *J Biol Chem* 1995;**270**:24548–24555.
32. Zhang D, Yang L, Ray A. Differential responsiveness of the interleukin-5 (IL-5) and IL-4 genes to transcription factor GATA-3. *J Immunol* 1998;**161**:3817–3821.
33. Andersson M, Andersson P, Pipkorn U. Topical glucocorticosteroids and allergen-induced increase in nasal reactivity: relationship between treatment time and inhibitory effect. *J Allergy Clin Immunol* 1988;**82**:1019–1026.
34. Horak F, Toth J, Hirschehr R, Marks B, Stubner UP, Jager S et al. Effect of continuous allergen challenge on clinical symptoms and mediator release in dust-mite-allergic patients. *Allergy* 1998;**53**:68–72.