

Original article

Recruitment of CD1a+ Langerhans cells to the nasal mucosa in seasonal allergic rhinitis and effects of topical corticosteroid therapy

Background: Local antigen presentation may be necessary for both primary and recall T-cell responses to grass pollen in hay fever patients. We examined the effect of seasonal allergen exposure on nasal mucosal antigen-presenting cell (APC) populations and the effects of topical corticosteroid therapy.

Methods: Nasal biopsies were collected from 46 grass pollen-sensitive seasonal rhinitis patients before the grass-pollen season. A second biopsy was collected during the pollen season, when patients had received 6 weeks' treatment with either fluticasone propionate (200 µg, twice daily) or placebo. Cell populations in biopsy sections were quantified by immunocytochemistry.

Results: Significant increases in submucosal and epithelial CD1a+ Langerhans cells, but not CD68+ macrophages or CD20+ B cells, were observed during the pollen season. Seasonal increases in CD1a+ Langerhans cells were inhibited by corticosteroid therapy.

Conclusions: Recruitment of CD1a+ Langerhans cells to the nasal mucosa during natural seasonal allergen exposure may contribute to local T cell responses. Topical corticosteroids may act, at least in part, by inhibiting effective allergen presentation to T cells through inhibition of recruitment of Langerhans cells to the nasal mucosa.

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We previously showed that allergen-induced late (1–24-h) nasal responses and seasonal allergic rhinitis caused by natural allergen exposure were accompanied by increased local expression of “Th2-type” cytokines, particularly interleukin-4 (IL-4) and IL-5 (1, 2). IL-4 favours IgE synthesis by B cells and may play a role in local heavy-chain switching within the nasal mucosa (1), whereas IL-5 is a specific eosinophil maturation, activation, and survival factor (3). The number of nasal mucosal CD25+ cells, presumed to be activated T cells, is also increased during the allergen-induced late response (4), and the majority of cells expressing mRNA encoding Th2-type cytokines following allergen provocation, at least at 24 h after challenge, are CD3+ T cells (5). The presence of activated memory T cells within the nasal mucosa of patients with allergic rhinitis suggests the possibility of local activation on allergen exposure. In order for this to occur, it is necessary for allergen to be processed by antigen-presenting cells (APC) and presented to mucosal T cells in a MHC-restricted fashion. It has been suggested that presenta-

tion of allergens in the airways by Langerhans cells favours secondary T-cell responses, whereas presentation by macrophages suppresses this response (6). It is therefore of considerable interest to document the populations of specialized APC in the nasal mucosa during natural seasonal exposure and examine the influence of treatment.

In the present study, we hypothesized that the recruitment of Langerhans cells to the nasal mucosa during the pollen season, which may facilitate local T-cell activation and cytokine production, would be inhibited by a clinically effective course of topical corticosteroid therapy, thereby contributing to down-regulation of the local T-cell response. To test these hypotheses, nasal biopsies were collected from asymptomatic allergic rhinitis patients before the start of the summer grass-pollen season. Under double-blind conditions, patients were then randomized to receive 6 weeks' treatment with either topical corticosteroid (fluticasone propionate 200 µg, twice daily) or placebo nasal spray. Further biopsies were subsequently col-

lected after 6 weeks, coinciding with the peak of the grass-pollen season. The numbers of specialized APC (CD1a + Langerhans cells, CD68 + macrophages, and CD20 + B cells) were assessed in nasal mucosal biopsies by immunocytochemistry.

Material and methods

Participants

Forty-six nonsmoking timothy grass pollen-sensitive patients were selected on the basis of

- 1) a history of moderate to severe seasonal allergic rhinitis for at least 2 years
- 2) a positive skin prick test (>5 mm wheal diameter on skin testing, in the presence of positive histamine and negative diluent controls) to timothy grass pollen extract (*Phleum pratense*, ALK, Hørsholm, Denmark).

Venous blood was also collected for measurement of total and allergen-specific IgE concentrations by ELISA. Patients were excluded if they had a history of perennial allergy or allergy to birch pollen, had received immunotherapy within the last 5 years, or had taken oral or topical glucocorticosteroids within the preceding 6 months. The study was performed with the approval of the hospital ethics committee and the written informed consent of all participants.

Study design and nasal biopsy

Patients were stratified into a "severe" group if they had symptoms for more than 10 weeks per year and a skin prick test result of >10 mm. All other patients were placed in the "moderate group". Both groups were then randomized to receive either a 6-week course of fluticasone propionate aqueous spray (Flixonase, Glaxo, Sweden), two sprays (200 µg) twice daily, or a placebo spray containing diluent. Their clinical response to fluticasone treatment during the pollen season has been reported previously (1, 2). Nasal biopsies were performed before the grass-pollen season when all patients were asymptomatic, and in the 2 weeks following the peak of the pollen season in Göteborg (peak mean weekly pollen count = 53 grains/m³, week ending 13 June). Local anaesthesia of the inferior nasal turbinate was achieved with 3% cocaine and 0.025% adrenaline, and a 2.5-mm biopsy was taken 10 min later with Gerritsma (7) forceps. Biopsies were snap frozen and stored at -80°C for immunohistology.

Immunohistochemistry

Immunohistology was performed on 6-µm cryostat sections (fixed for 7 min in 60:40 acetone:methanol), with the modified alkaline phosphatase-anti-alkaline phosphatase method, as previously described (8). Monoclonal antibodies recognizing CD68 (macrophages) and CD20 (B cells), were all obtained from Dako Ltd, High Wycombe, UK. Monoclonal antibody recognizing CD1a was obtained from the 66IIC7 clone. The specificity of the 66IIC7 clone for the CD1 antigen (Langerhans cells) has been discussed elsewhere (9).

Statistical analysis

Within groups, pre- and peak-season counts were compared by the Wilcoxon matched-pairs signed rank test. Seasonal changes ("peak-season" values minus "preseason" values) in fluticasone- and placebo-treated groups were compared by the Mann-Whitney U-

test. All analyses were performed with the aid of a commercial software package (Minitab, Inc., PA, USA), and *P* values less than 0.05 were considered significant.

Results

Randomization resulted in 23 patients (16 male) receiving fluticasone treatment and 23 receiving a placebo nasal spray (14 male). The groups were well matched for age, mean $\pm$ SD 33.1 ± 10.7 years for the placebo group and 32 ± 8.9 years for the fluticasone group. Total and specific IgE levels were comparable for both groups. The placebo group median total IgE was 70 IU/ml (interquartile range 25,130), and mean RAST (timothy grass; score 0–5) was 3.2 ± 0.8 . The fluticasone group median total IgE was 50 IU/ml (interquartile range 25,120), and mean RAST was 2.9 ± 0.8 . In both fluticasone- and placebo-treated patients, increased symptoms of allergic rhinitis (as assessed by a weekly nasal visual analogue score) coincided with increases in timothy grass-pollen counts over the months of May to July (2). Fluticasone-treated patients showed markedly reduced symptoms over this observation period as compared with placebo-treated controls (Fig. 1, $P=0.003$).

Numbers of CD1a + Langerhans cells, CD68 + macrophages, and CD20 + B cells in nasal mucosa before and during pollen season: effects of fluticasone treatment

Placebo-treated patients showed statistically significant seasonal increases in the numbers of submucosal CD1a + Langerhans cells ($P=0.005$, Table 1, Fig. 2a). In contrast, the numbers of CD1a + Langerhans cells *decreased* in the fluticasone-treated patients ($P=0.03$, Table 1, Fig. 2a), and the overall seasonal changes were significantly different statistically between the two groups ($P=0.0004$, Fig. 2a). Similarly, the numbers of intraepithelial CD1a + Langerhans cells increased in the placebo group during the pollen season ($P=0.03$, Table 1, Fig. 2b), while intraepithelial CD1a + Langerhans cell numbers did not change in the fluticasone-treated group during the peak pollen season (Table 1). The seasonal changes in numbers of intraepithelial CD1a + cells were significantly different between the two groups ($P=0.03$, Fig. 2b).

The number of submucosal and intraepithelial CD68 + macrophages did not change with increased allergen exposure during the pollen season in either the placebo- or fluticasone-treated group (Table 1). Fluticasone treatment had no effect on submucosal or intraepithelial CD68 + macrophage numbers (Table 1).

Likewise, the numbers of submucosal CD20 + B cells did not change with exposure to grass pollen in either the placebo- or fluticasone-treated group

Table 1. Median cell counts per square millimetre in nasal submucosa and nasal epithelium

	Placebo			Fluticasone			P values
	Preseason	Peak season	Median differences (95% CI)	Preseason	Peak season	Median differences (95% CI)	
Submucosa							
CD1a	0	2.5	2.5 (0.5, 11.8)*	0	0	-0.25 (-1.0, 0)†	0.0004
CD68	91.2	117.0	25.8 (-19.0, 76.2)	70.0	80.0	13.4 (-23.3, 42.2)	NS
CD20	19.5	26.0	4.3 (-13.5, 18.7)	9.8	11.0	-5.8 (-29.6, 5.3)	NS
Epithelium							
CD1a	0	11.4	8.1 (0, 32.1)†	0	0	0 (-3.15, 0)	0.03
CD68	48.0	49.7	15.1 (-23.3, 61.6)	64.4	33.4	-26.8 (-56.7, 5.6)	NS
CD20	0	0	-5.6 (-12.6, 0)†	0	0	-3.9 (-13.4, 0)	NS

CI: confidence interval; NS: not significant. Between-group analysis was performed with Mann-Whitney U-test. Within-group analysis was performed with Wilcoxon's matched-pairs, signed-ranks test. * $P < 0.005$. † $P < 0.05$.

(Table 1). In placebo-treated patients, grass-pollen exposure was associated with a small reduction in the numbers of epithelial CD20+ B cells, although median values were zero both in and out of season ($P = 0.02$, Table 1). Fluticasone-treated patients also showed a trend to a reduction in epithelial CD20+ B cells during the pollen season ($P = 0.13$, Table 1). Seasonal changes in the numbers of intraepithelial CD20+ B cells were not significantly different between the placebo- and fluticasone-treated groups ($P = 0.91$).

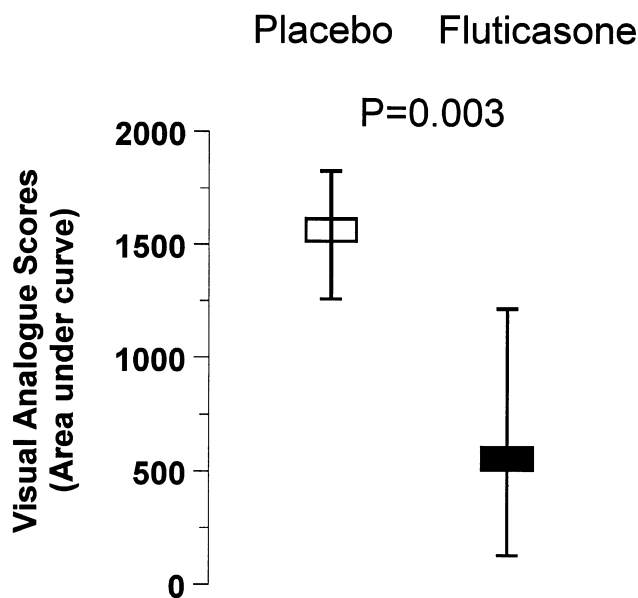


Figure 1. Seasonal rhinitis symptoms in placebo- or fluticasone-treated patients during natural exposure to timothy grass pollen (*Phleum pratense*). Visual analogue scores (in response to the question "How has your hay fever been this week?", score 1–10, 1 = no symptoms) were subjected to "area under curve" analysis over pollen season for individual patients. Bars represent median ($\pm$ interquartile range) of these values in placebo- or fluticasone-treated groups.

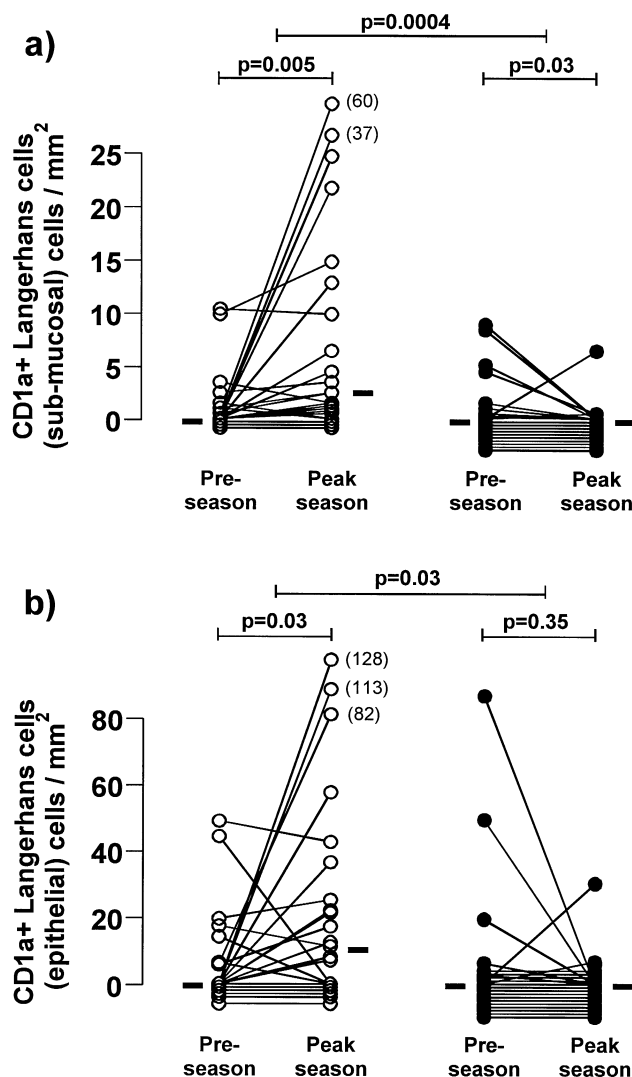


Figure 2. Effect of fluticasone on numbers of CD1a+ Langerhans cells in a) nasal submucosa and b) nasal epithelium. Data shown are for baseline ("preseason") and during exposure to natural grass-pollen allergen ("peak-season"), in placebo- (open circles) fluticasone- (closed circles) treated allergic rhinitis patients. Lines represent median values.

Discussion

Hay-fever symptoms induced by seasonal exposure to grass pollen were accompanied by significant increases in the numbers of CD1a+ Langerhans cells, within both the epithelium and the submucosa, confirming an earlier report (10). We further showed that these seasonal increases in professional antigen-presenting Langerhans cells were completely inhibited by 6 weeks' treatment with the potent topical nasal corticosteroid fluticasone propionate. In contrast, we observed no seasonal increases in CD68+ macrophages or CD20+ B cells and no effect of topical corticosteroid on these alternative APC.

Although the precise role of mucosal APC in the immune response to allergens remains a matter of speculation, two distinct phases have been proposed. First, naive allergen-specific T cells become sensitized by APC, presumed to be Langerhans cells, which trap allergens in mucosal tissues and migrate to regional lymph nodes. Second, allergen-specific memory T cells circulate in the airways and are activated locally when inhaled allergens are presented by APC *in situ* (11). According to this proposal, it is likely that in allergic rhinitis and asthma local professional APC are important in allergen-triggering of recall responses during natural seasonal exposure to grass pollen. The resulting activation of mucosal memory CD4+ T cells producing IL-4 and IL-5 has the potential to drive local effector responses characterized by mucosal eosinophilia (2) and B-cell IgE synthesis (1). During ongoing allergic disease, it is also likely that there is a continued sensitization of "new" naive T cells, caused partly by migration of Langerhans cells from the mucosa to the draining regional lymph nodes.

In the present study, the absence of any seasonal increases in the numbers of nasal mucosal B cells and macrophages excludes the possibility of these cells upregulating their APC activity through an increase in numbers alone. On the other hand, these data certainly do not exclude the possibility that individual B cells and macrophages may become more effective APC on allergen exposure; for example, as a consequence of activation or passive sensitization with IgE synthesized during the pollen season (12). In the case of B cells, the present study is consistent with our previous report that the numbers of B cells in nasal biopsies do not change 24 h after experimental allergen challenge to the nose (13). Our finding that macrophage numbers do not increase during the pollen season confirms our previous finding in monoallergic grass-sensitive patients (8). It is, however, at variance with some previous studies performed either on nasal biopsies (14) or nasal scrapings (15) from patients with seasonal allergic rhinitis or following repeated daily allergen challenge (11) which did identify increases in macrophages. A possible explanation may reside in differences in the

chronicity or intensity of pollen exposure, or the presence of various other allergies that were not present in our patients. It is noteworthy that the results are in agreement with findings in atopic asthma, where studies have indicated that numbers of macrophages within the bronchial mucosa are not increased compared to normal control subjects (16).

There is evidence that both B cells and macrophages have the capacity to present allergen and activate allergen-specific T cells in the respiratory mucosa of patients with rhinitis or asthma. In the case of B cells this evidence is, however, entirely circumstantial: reports describing IgE production in the nasal mucosa of allergic rhinitis patients (1, 13, 17) indicate local availability of IL-4/IL-13 and CD40L, and therefore possible B-T-cell collaboration within the mucosa. Furthermore, studies conducted on human peripheral blood B and T cells have also demonstrated that B cells can efficiently present allergen to T cells and may preferentially stimulate IL-4 synthesis as compared with monocytes (18). The properties of nasal mucosal macrophages as APC have also not been tested directly in functional assays, although the recent demonstration of high-affinity IgE receptor alpha subunit (FcεRIα) expression by these cells (19) suggests the possibility of IgE-mediated antigen processing and presentation. Indeed, some studies have suggested that alveolar macrophages from asthmatic patients show an enhanced ability to activate allergen-specific T cells and promote Th2-type cytokine production compared to macrophages from control subjects (20, 21), whereas other investigators have proposed that macrophages suppress T cell responses to inhaled antigens (6).

In contrast to B cells and macrophages, we did observe significant increases in the numbers of epithelial and submucosal Langerhans cells during the pollen season. These increases were inhibited by a 6-week course of treatment with fluticasone nasal spray. These data are consistent with previous studies reporting increased epithelial CD1a+ Langerhans cells in seasonal allergic rhinitis patients during the pollen season (10) and after repeated daily allergen provocation for 2 weeks during the winter months (22), and decreased epithelial and submucosal CD1a+ Langerhans cells in perennial rhinitis patients treated with nasal corticosteroids (23). Our data are, however, the first to show a direct effect of topical corticosteroids on numbers of CD1a+ Langerhans cells during natural seasonal exposure to grass pollen. Collectively, the findings have important implications for our understanding of the allergic immune response in the target organ. Langerhans cells are potent APC. In one animal model, lung Langerhans cells were selectively depleted from the lungs of thymidine-kinase transgenic mice by application of ganciclovir (24) after the animals had been sensitized with allergen. The secondary response to inhaled allergen, normally characterized by Th2

cytokine expression and eosinophilia, was abrogated in these mice. Furthermore, clusters of Langerhans cells and T cells exist within the human nasal submucosa (25). These findings suggest, as discussed above, that the role of Langerhans cells extends beyond sensitization to the maintenance of mucosal allergic inflammation through local presentation of inhalant allergens to memory Th2 T cells.

A number of mechanisms could account for the effects of corticosteroids in suppressing the seasonal increases in the numbers of nasal mucosal CD1a+ Langerhans cells. First, corticosteroids may inhibit the production of cytokines such as GM-CSF (26) and IL-4 (1), which favour differentiation of dendritic cells from precursors (27, 28). Second, expression of CC chemokines in the nasal mucosa of allergic rhinitic patients can be inhibited by topical corticosteroids (29, 30). The responsiveness of dendritic cells and/or their precursors to certain CC chemokines *in vitro* (31, 32) suggests that these mediators could be involved in Langerhans cell recruitment to the nasal mucosa *in vivo*. Third, there is also the possibility that corticosteroids downregulate expression of surface CD1a+ molecules by Langerhans cells, as previously described for murine epidermal

Langerhans cells (33). Fourth, in a rodent model, corticosteroids were observed to deplete dendritic cells in the tracheal mucosa by inducing apoptosis (34). Finally, it is important to note that in addition to their effects on Langerhans cell numbers *in vivo*, corticosteroids also have the capacity to inhibit antigen uptake and processing by dendritic cells *in vitro* (35).

In summary, we have shown that seasonal allergic rhinitis coincides with epithelial and submucosal infiltration by CD1a+ Langerhans cells, but not CD68+ macrophages or CD20+ B cells. This response was suppressed in patients receiving topical corticosteroid therapy, suggesting that this form of treatment may act, at least in part, by downregulating presentation of aeroallergens to T cells by CD1a+ Langerhans cells.

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