

Increased number of CD34⁺ cells in nasal mucosa of allergic rhinitis patients: inhibition by a local corticosteroid

S. Sergejeva, C. Malmhäll, J. Lötvald and T. Pullerits

The Lung Pharmacology Group, Department of Respiratory Medicine and Allergology, Institute of Internal Medicine, Göteborg University, Gothenburg, Sweden

Summary

Background Eosinophils develop from CD34⁺ haematopoietic progenitor cells. Allergen exposure in susceptible individuals is known to induce a local eosinophilic inflammation, but the effect on progenitor cells is much less understood.

Objective We aimed to evaluate how allergen exposure affects the number of tissue CD34⁺ cells and CD34⁺ eosinophils in allergic rhinitis (AR) patients and whether any such effect is influenced by local corticosteroid treatment. Also, we evaluated changes in the number of CXC receptor 4-positive cells (CXCR4⁺), since the CXCR4 ligand (stromal cell-derived factor-1 (SDF-1)) is a potent chemoattractant for haematopoietic progenitors.

Methods In a double-blind, randomized study, pollen-sensitized AR patients were treated with a nasal corticosteroid fluticasone propionate (FP, 200 µg/day) or placebo throughout the pollen season. Nasal biopsies were taken before and during the season. CD34 and CXCR4 were stained using immunohistochemistry.

Results The pollen season significantly increased the number of CD34⁺ cells, CD34⁺/CXCR4⁺ cells and CD34⁺ eosinophils in placebo-treated patients, but not in FP-treated patients. The mean pollen season-induced increase in CD34⁺ cells, CD34⁺/CXCR4⁺ cells and CD34⁺ eosinophils in FP-treated patients was lower compared with placebo-treated patients.

Conclusion A pollen season increases the number of CD34⁺ cells in nasal tissue accompanied by an increase in the number of CD34⁺/CXCR4⁺ haematopoietic progenitors and also the number of CD34⁺ eosinophils in subjects with AR. Treatment with a local corticosteroid provides protection against this pollen-induced increase in tissue CD34⁺ cells and CD34⁺ eosinophils possibly via inhibition of allergen-induced CXCR4-mediated recruitment of CD34⁺ haematopoietic progenitors into airways and their further differentiation into eosinophils within the tissue.

Keywords allergic rhinitis, CD34⁺ cells, haematopoietic progenitor, local corticosteroid

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Introduction

Local airway inflammation in allergic rhinitis (AR) is characterized by the infiltration of mucosa with eosinophils. Products from eosinophils such as platelet-activating factor, leukotrienes and eosinophil granule proteins may account for the prolonged nasal congestion and mucus secretion observed during natural allergen exposure in grass-pollen season. Increased number of airway eosinophils is the result of increased eosinophil differentiation within bone marrow and their recruitment via the circulation into airways [1–3]. Eosinophils develop from the CD34 antigen bearing haematopoietic progenitor cells, which is stage-specific rather than lineage-specific leucocyte differentiation antigen. Its expression appears at the highest density on earlier progenitors,

decreases progressively with cell maturation and is lost on terminally differentiated cells. Apart from increased eosinophil number, atopic subjects have increased number of CD34⁺ cells in bone marrow, blood [3] and airways [4]. Importantly, airway CD34⁺ cells differentiate into eosinophils following *ex vivo* stimulation with allergen [5]. These data suggest that eosinophil differentiation may also occur within the airways. To date, there are no data available regarding allergen-induced changes in tissue progenitor cells in airways of atopic patients. Furthermore, it is not clear whether tissue CD34⁺ cells are airway residing cells or are recruited into airways in response to allergen exposure.

Local corticosteroids are used as anti-inflammatory treatment in allergic airway diseases. They are known to diminish the number of airway eosinophils as well as progenitor cells in blood and bone marrow of atopic asthmatics [6, 7]. To date, only one study has evaluated the effect of a local corticosteroid on airway progenitor cells, demonstrating an increase in the expression of CD34 antigen in nasal polyp tissue following corticosteroid treatment [8]. However, the effect of a local

Correspondence: Teet Pullerits, The Lung Pharmacology Group, Department of Respiratory Medicine and Allergology, Institute of Internal Medicine, Göteborg University, Guldhedsgatan 10A, 413 46 Gothenburg, Sweden. E-mail: teet.pullerits@lungall.gu.se

corticosteroid on allergen-induced CD34⁺ haematopoietic progenitor cells in airways has not been reported.

We therefore aimed to evaluate how seasonal allergen exposure affects the number of nasal mucosal CD34⁺ cells and CD34⁺ eosinophils in AR patients. Also, we evaluated changes in the expression of CXCR4 on CD34⁺ cells, since CXCR4 ligand (stromal cell-derived factor-1 (SDF-1)) is the most potent chemoattractant for haematopoietic progenitors [9] and finally how these effects might be influenced by a local corticosteroid treatment.

Methods

Nasal biopsy samples were obtained from a clinical study, described in detail in a previous publication [10]. Briefly, grass-pollen sensitized AR patients were treated with either a nasal corticosteroid fluticasone propionate (FP; 200 µg/day) ($n = 13$; median age 30, range 18–40 years) or respective placebo ($n = 16$; median age 30, range 15–48 years) for 50 days throughout the grass-pollen season, starting approximately 2 weeks before the expected beginning of the season. Nasal biopsy samples were obtained on two occasions during the study. The pre-season samples were taken 1–2 months before initiation of treatment and the in-season samples during the peak of the grass-pollen season. The study was approved by the local Ethics Review Committee and all patients gave their written informed consent.

Immunohistochemical detection of CD34 antigen with luxol fast blue counterstaining

Nasal biopsy samples were obtained and processed as previously described [10]. Tissue sections were fixed with 2% formaldehyde and unspecific binding was blocked with 5% rabbit serum (DAKO Corporation, Glostrup, Denmark). The slides were then incubated with a monoclonal mouse anti-human CD34 class II antibody (QBEnd 10, DAKO) or its isotype control, mouse IgG₁κ (MOPC 21, Sigma Aldrich Sweden AB, Tyresö, Sweden) for 1 h in room temperature and subsequently incubated for 45 min with alkaline phosphatase-conjugated rabbit anti-mouse IgG₁ antibody (Zymed Laboratories, San Francisco, CA, USA). Bound antibodies were visualized with the Vector[®] red alkaline phosphatase substrate kit (Vector Laboratories Inc., Burlingame, CA, USA). Lastly, to detect eosinophilic granulation, counterstaining with luxol fast blue (LFB) was performed.

The numbers of CD34⁺ cells were counted within 250 µm of subepithelium and expressed as total number per 1 mm² of tissue area. Since apart from haematopoietic progenitor cells, CD34 antigen is also expressed on small-vessel endothelial cells [11], only cells with phenotypical features of haematopoietic progenitors (round cells with high nucleus-to-cytoplasm ratio) were included. Vascular endothelial cells showed diffuse cytoplasmic immunostaining for CD34 antigen, but were easily excluded based upon their distinct morphology.

Immunohistochemical detection of CD34 antigen and CXCR4 receptor

The double staining was performed as a sequential method based on two individually performed complete staining

procedures. Tissue sections were fixed with 4% zinc formaldehyde (Histolab Products AB, Göteborg, Sweden) and endogenous biotin was blocked with biotin blocking system (DAKO) followed by incubation for 1 h with a monoclonal mouse anti-human CXCR4 antibody (12G5, R&D Systems Inc., Abingdon, UK) or its isotype control, mouse IgG_{2a}κ (G155–178, BD Biosciences Europe, Erembodegem, Belgium). The slides were then incubated with a biotinylated rabbit F(ab')₂ anti-mouse Ig (DAKO) for 1 h followed by β-galactosidase-conjugated streptavidin for 45 min. Bound antibodies were visualized with the β-galactosidase staining set (Roche Diagnostics GmbH, Mannheim, Germany) for 40 min at 37 °C. Sections were incubated overnight at +4 °C in 10% rabbit serum. Detection of CD34 antigen was performed as described above. Mayer's Haematoxylin (Sigma Aldrich Sweden AB) was used for counterstaining. Control stainings were performed using the above-described procedures with following combinations of antibodies and/or isotype controls: (1) isotype controls for both anti-CXCR4 and anti-CD34 antibodies; (2) isotype control for anti-CXCR4 antibody and anti-CD34 antibody; (3) anti-CXCR4 antibody and isotype control for anti-CD34 antibody. Numbers of CD34⁺/CXCR4⁺ haematopoietic cells were counted within 250 µm of subepithelium and are expressed as relative number from total counted CD34⁺ cells.

Data analysis

Data are presented as means with SEM. Wilcoxon signed ranks test was used to analyse pollen-season induced changes in the numbers of CD34⁺ cells and CD34⁺ eosinophils within the groups. The Mann–Whitney *U*-test was employed for comparison of these changes (Δ change) between the groups. $P < 0.05$ was considered statistically significant.

Results

The data on the clinical outcome of the study and on the local nasal eosinophilic inflammation (tissue EG2⁺ eosinophil counts) in these samples are previously presented [10].

CD34⁺ cells in nasal biopsies

There was no statistically significant difference in the numbers of CD34⁺ cells between the treatment groups in pre-season nasal biopsy samples. The grass-pollen season induced an increase in the numbers of CD34⁺ cells in placebo-treated patients (from 48 ± 6 to 68 ± 7 cells/mm², $P = 0.036$). No such increase was present in patients treated with nasal FP (from 54 ± 4 to 45 ± 6 cells/mm², $P = 0.08$). The mean pollen-season-induced change (Δ change) in CD34⁺ cells was significantly lower in FP-treated patients compared with placebo-treated patients (-9 vs. $+20$ cells/mm², respectively, $P = 0.018$, Fig. 1).

CD34⁺ eosinophils in nasal biopsies

Immunohistochemical staining for CD34 antigen with LFB counterstaining in the nasal biopsy of AR patient is illustrated in Fig. 2. The pollen-season induced a significant

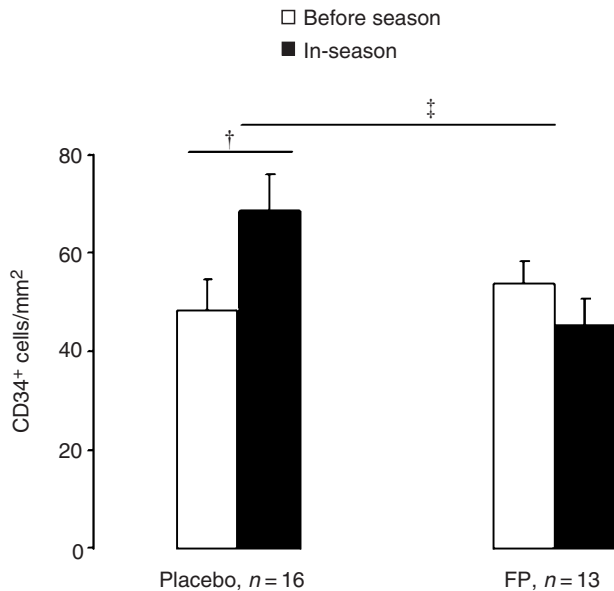


Fig. 1. The pollen-season-induced change in the number of subepithelial CD34⁺ cells in nasal biopsies of patients with allergic rhinitis (AR) treated with local fluticasone propionate (FP) or placebo. †Wilcoxon signed ranks, $P < 0.05$ for changes in the number of CD34⁺ cells from pre- to in-season samples within groups; ‡Mann–Whitney U -test, $P < 0.05$ for differences in Δ change in CD34⁺ cells between groups.

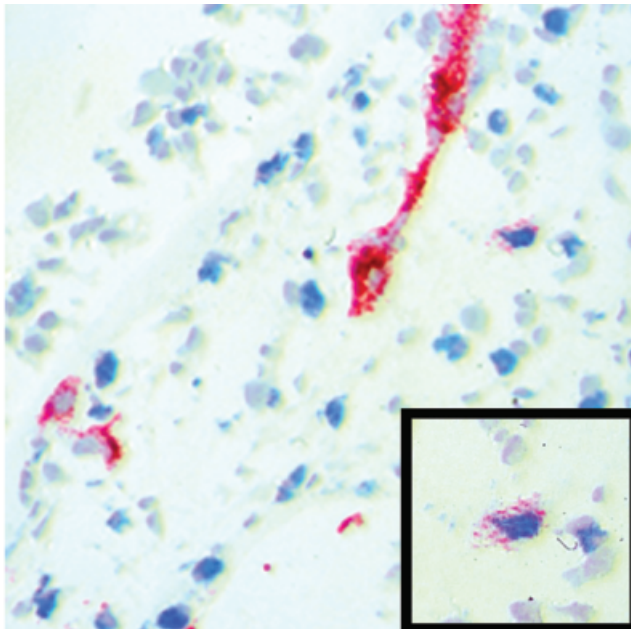


Fig. 2. Immunohistochemical staining for CD34 antigen (red stain) with luxol fast blue counterstaining (green stain) in the nasal biopsy of allergic rhinitis (AR) patient ($\times 400$).

increase in the numbers of CD34⁺ eosinophils in placebo-treated (from 0.25 ± 0.25 to 3.5 ± 1.4 cells/mm², $P = 0.034$), but not in FP-treated patients (from 0.8 ± 0.6 to 0.3 ± 0.3 cells/mm², $P = 0.28$). The Δ change in the CD34⁺ eosinophils in FP-treated patients was significantly lower compared with placebo-treated patients (-0.5 vs. 3.3 cells/mm², respectively, $P = 0.046$, Fig. 3).

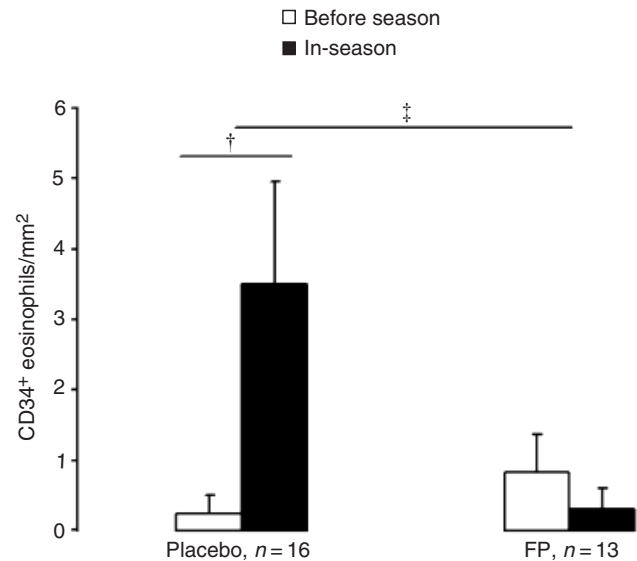


Fig. 3. The pollen-season-induced change in the number of subepithelial CD34⁺ eosinophils in nasal biopsies of patients with allergic rhinitis (AR) treated with local fluticasone propionate (FP) or placebo. †Wilcoxon signed ranks, $P < 0.05$ for changes in the number of CD34⁺ eosinophils from pre- to in-season samples within groups; ‡Mann–Whitney U -test, $P < 0.05$ for differences in Δ change in CD34⁺ eosinophils between groups.

CD34⁺/CXCR4⁺ haematopoietic cells in nasal biopsies

Immunohistochemical staining for CD34 antigen and CXCR4 receptor in the nasal biopsy of AR patient is illustrated in Fig. 4. There was no statistically significant difference in the numbers of CD34⁺/CXCR4⁺ haematopoietic cells between the treatment groups in pre-season nasal biopsy samples. The grass-pollen-season induced an increase in the relative numbers of CD34⁺/CXCR4⁺ haematopoietic cells in placebo-treated patients (from $9.6 \pm 2.0\%$ to $25.0 \pm 2.7\%$ of total CD34⁺ cells, $P = 0.001$). No such increase was present in patients treated with nasal FP (from $10.2 \pm 2.7\%$ to $7.3 \pm 2.8\%$ of total CD34⁺ cells, $P = 0.3$). The Δ change in the relative number of CD34⁺/CXCR4⁺ haematopoietic cells was significantly lower in FP-treated patients compared with placebo-treated patients (-3% vs. 15% , respectively, $P = 0.0007$, Fig. 5).

Discussion

The present study shows that in subjects with AR, the *in vivo* exposure to allergen during a grass-pollen season increases the number of nasal mucosal CD34⁺ cells. As a novel mechanism, we also demonstrate concomitant up-regulation of CXCR4 expression within the CD34⁺ cell population. Furthermore, also the number of CD34⁺ eosinophils in nasal mucosa is increased following exposure to allergen. A local corticosteroid, FP, provides protection against this pollen-induced increase in tissue CD34⁺ cells.

The increased number of CD34⁺ cells in bronchial biopsies has been demonstrated in atopics compared with healthy individuals [4]. However, to date, no information is available in the literature regarding the relationship between tissue progenitor cells and allergen exposure *in vivo*. Here, we show that a pollen season induces an increase in the number of

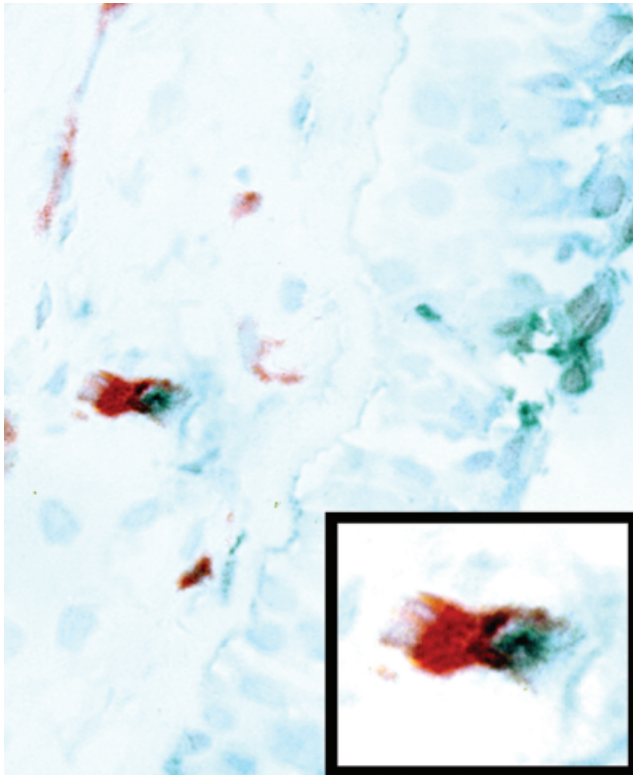


Fig. 4. Immunohistochemical staining for CD34 antigen (red stain) and CXCR4 receptor (green stain) in the nasal biopsy of allergic rhinitis (AR) patient ($\times 400$).

CD34⁺ cells in nasal mucosa. Unlike most leucocyte subsets, haematopoietic progenitor cells have restricted responsiveness to chemokines. In fact, there is only one known potent chemoattractant for progenitor cells, namely SDF-1, which is constitutively expressed in a variety of cell types [9]. SDF-1 elicits rapid and direct *in vitro* chemotaxis of human bone marrow and blood myeloid lineage-committed CD34⁺ cells [12]. The only known receptor for SDF-1 is CXCR4 [13]. *In vitro* experiments have shown that eosinophils that have differentiated from blood CD34⁺ cells retain CXCR4 receptor up to the stage of mature eosinophil [14]. Furthermore, bronchoalveolar lavage fluid eosinophils have enhanced expression of CXCR4 compared with blood eosinophils, suggesting that expression of this receptor has a role in recruitment of eosinophils into airways [15]. Also, elevated CXCR4 mRNA expression in nasal scrapes has been recently reported in symptomatic AR patients [16]. In our study we show that allergen exposure increases the number of CD34⁺/CXCR4⁺ haematopoietic progenitors in nasal mucosa. The proportion of CD34⁺ haematopoietic cells expressing CXCR4 was increased from 10% to 25% after allergen exposure. Taking into account that the increase in mucosal CD34⁺ cells was of a similar magnitude (20%) and that the CXCR4 ligand causes chemotaxis of haematopoietic progenitors, it can be hypothesized that the observed increase in the tissue CD34⁺ cells is the result of the allergen-induced CXCR4-mediated recruitment of CD34⁺ haematopoietic cells.

A study performed on allergic nasal mucosa explants suggested a concept of local eosinophil differentiation from CD34⁺ cells [5]. This concept was based upon increase in the number of MBP⁺ cells concomitant with decrease in number

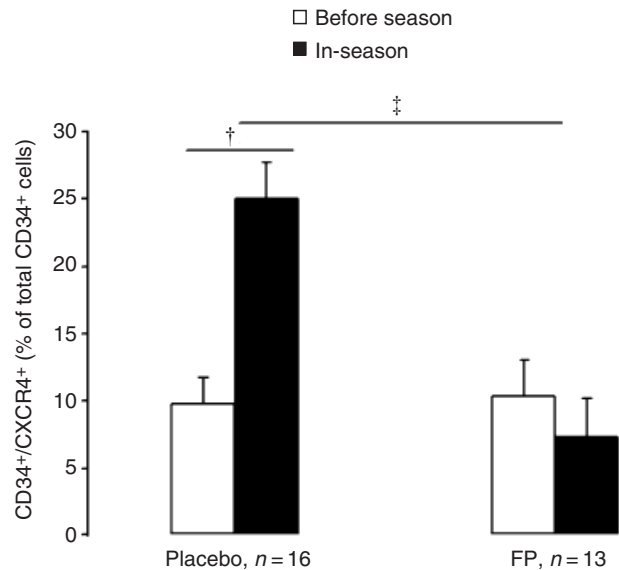


Fig. 5. The pollen-season-induced change in the number of subepithelial CD34⁺/CXCR4⁺ cells in nasal biopsies of patients with allergic rhinitis (AR) treated with local fluticasone propionate (FP) or placebo. †Wilcoxon signed ranks, $P < 0.05$ for changes in the number of CD34⁺/CXCR4⁺ cells from pre- to in-season samples within groups; ‡Mann-Whitney *U*-test, $P < 0.05$ for differences in Δ change in CD34⁺/CXCR4⁺ cells between groups.

of CD34⁺/IL-5R α ⁺ cells in response to *ex vivo* stimulation with allergen. In the process of the eosinophil differentiation from pro-myelocyte stage onwards, eosinophilic granules become increasingly conspicuous in the cytoplasm. Hence, in our experiment we utilized immunohistochemistry for CD34 antigen together with LFB counterstaining, which allowed us to identify the CD34 antigen on eosinophilic cells. Using this method we show that allergen exposure increases the number of CD34⁺ eosinophils in nasal mucosa, arguing for allergen-induced local eosinophil differentiation.

Local corticosteroids decrease the baseline bone marrow [6] and allergen-induced blood progenitor numbers [7] in atopic asthmatics, suggesting that the anti-inflammatory effect of corticosteroids is at least in part because of an effect on the production and release of progenitor cells from bone marrow. Furthermore, it has been shown that corticosteroids inhibit differentiation of CD34⁺ cells into eosinophils in nasal polyps [8]. Here, we show that a local corticosteroid prevents allergen-induced increase in the number of mucosal CD34⁺ cells in the nasal tissue accompanied by inhibition of allergen-induced increase in CXCR4-positive CD34⁺ haematopoietic cells. Since the CXCR4 is the receptor for the potent haematopoietic progenitor cell chemoattractant (SDF-1), it is possible that corticosteroids attenuate the recruitment of CD34⁺ cells into airways via reduced expression of CXCR4. In addition, FP decreases the number of CD34⁺ cells with eosinophilic granulation, arguing that topical corticosteroids prevent allergen-induced local differentiation of CD34⁺ haematopoietic cells into eosinophils. These findings together therefore argue that local corticosteroid inhibits both allergen-induced recruitment of CD34⁺ haematopoietic progenitors into airway tissue as well as their further local differentiation into eosinophils.

In conclusion, we show that allergen exposure induces recruitment of CD34⁺ haematopoietic cells into the airways

in vivo, where one of the possible mechanisms is CXCR4-mediated CD34⁺ cell chemotaxis. Furthermore, allergen exposure induced an increase in the number of mucosal CD34⁺ cells with an eosinophilic phenotype, arguing that allergen drives eosinophil differentiation within tissue *in vivo*. In addition, a local corticosteroid inhibits both allergen-induced CXCR4-mediated recruitment of CD34⁺ haematopoietic cells and their further differentiation towards eosinophils within the inflamed tissue.

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