

Short communication

Generation of clusters of free eosinophil granules (Cfegs) in seasonal allergic rhinitis

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Generation of clusters of free eosinophil granules (Cfegs), through lysis of eosinophils, has recently been proposed as a major paradigm for ultimate activation of airway mucosal eosinophils. In the present study involving patients with seasonal allergic rhinitis, we have investigated whether generation of Cfegs in the nasal mucosa is a feature of allergic rhinitis. Nasal mucosal biopsies were obtained before and late in a birch-pollen season, and were subjected to histochemical staining of eosinophil peroxidase. In biopsies obtained before the pollen season, a few, intact eosinophils were observed, and Cfegs were scarce. In biopsies taken during the pollen season, the numbers of eosinophils were increased about 10-fold ($P < 0.05$) and the Cfegs about 25-fold ($P < 0.05$). We conclude that generation of Cfegs is a significant feature of seasonal allergic rhinitis and that this process represents the ultimate activation of mucosal eosinophils in this disease.

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Generation of extracellular eosinophil granules (clusters of free eosinophil granules [Cfegs]) may reflect the ultimate activation of eosinophils, through inflammatory stimulus-induced lysis of these cells (1, 2). This notion is partly based on *in vivo* experiments demonstrating numerous Cfegs in whole-mount preparations (avoiding artifacts) of the guinea-pig airway mucosa (3, 4). Furthermore, eosinophil lysis and number of Cfegs have been demonstrated to increase promptly after common airway challenges involving epithelial injury and allergen exposure (3–5). The Cfegs consist of granules in different stages of dissolution, and they are intriguingly distributed close to various target cells and target end-organs in the airway mucosa (1–5). Cfegs are also a conspicuous feature of asthmatic sputa as examined in whole-mount preparations after prompt fixation of spontaneously delivered sputum samples (5). Similarly, nasal discharge (whole-mount preparations) spontaneously delivered within 20 min after allergen challenge also contains many Cfegs (5). Studies of surgically removed nasal polyps further demonstrate Cfegs in whole-mount preparations of human airway tissue (5). In the present study involving patients with seasonal allergic rhinitis, we report on the occur-

rence of eosinophils and Cfegs in nasal mucosal biopsies obtained before and late in a birch-pollen season.

Material and methods

Study design

Nasal biopsies were obtained from patients with seasonal allergic rhinitis before and late in the Swedish birch-pollen season of 1995. The numbers of eosinophils and Cfegs (per defined area) were examined. Furthermore, nasal symptoms were scored. The study was blinded for histologic analysis.

Patients

The subjects were recruited from the Department of Otorhinolaryngology, Head and Neck Surgery, University Hospital, Lund, Sweden. Eight patients with seasonal allergic rhinitis over the age of 18 were included. Inclusion criteria were history of strictly seasonal allergic rhinitis and a positive skin prick test to birch-pollen allergen. Exclusion criteria were a history of chronic nasal disease other

than seasonal allergic rhinitis, positive skin prick tests to perennial allergens, drug treatment, and smoking. The study was approved by the local ethics committee, and informed consent was obtained.

Nasal symptoms

Rhinorrhea, blockage, and sneezes were scored daily by the subjects on a four-point scale: score 0: no, 1: mild, 2: moderate, and 3: severe symptoms.

Nasal biopsies

Nasal biopsies were obtained before and late in (study days 27 and 28) the pollen season. Topical anesthesia was applied with a solution of tetracain (20 mg/ml) and epinephrine (0.1 mg/ml) delivered by a nasal spray device. Nasal biopsies were taken from the inferior aspects of the inferior nasal turbinate about 0.5 cm from its anterior margin. The Gerritsma punch forceps with the 3-mm punch was used for this purpose (6). Nasal biopsies before the pollen season were obtained from the right nasal cavities, whereas biopsies from the left nasal cavities were obtained late in the pollen season.

Histochemical staining of eosinophil peroxidase (EPO) and histologic evaluation

Eosinophil secondary granules were visualized in conventional cryostat sections (10 μ m) by enzyme histochemical staining of cyanide-resistant EPO (3). Immediately after excision, biopsies were placed in Stefanini fixative (PBS buffer, pH 7.6, supplemented with 4% formaldehyde and picric acid [3]), overnight at 4°C. Next the specimens were rinsed thoroughly in PBS buffer and incubated for 8 min in PBS supplemented with 3,3-diamino-benzidine-tetrahydrochloride (75 μ g/100 ml), 30% H_2O_2 (0.3 ml/100 ml), and NaCN (50 mg/100 ml) at room temperature. Then the samples were rinsed in water, mounted in Kaiser's medium, and examined under a bright-field microscope. The specificity of this staining has been validated (3). Areas subjected to mechanical stress were excluded from evaluation. The total number of (intact) eosinophils and Cfegs (three or more granules together) were counted in two sections from each biopsy. All quantifications were carried out in a blinded fashion.

Statistics

Differences in symptom scores as well as numbers of eosinophils and Cfegs were examined by Wilcoxon's signed rank test. Data are presented as means $\pm$ SEM.

Results

Eight patients were enrolled in the study. One patient failed to follow the protocol due to upper-respiratory-tract infection at the planned time of entry and was excluded. The birch-pollen counts, recorded about 10 miles from the study area, presented moderate pollen levels. Nasal symptoms recorded during the study period accordingly demonstrated a significant increase from study 17 and throughout the study period ($P < 0.05$) (Fig. 1).

In biopsies obtained before the pollen season, few eosinophils were observed. These eosinophils were mostly intact, and almost no Cfegs were present (Fig. 2). In biopsies taken during the pollen season, the numbers of eosinophils were increased about 10-fold ($P < 0.05$) and the Cfegs about 25-fold ($P < 0.05$) (Figs. 2 and 3). The Cfegs were distributed throughout the mucosal tissue.

Discussion

The present study, involving patients with seasonal allergic rhinitis, has confirmed the seasonal increase in nasal mucosal eosinophils in this disease (7), and has demonstrated a marked increase in the number of Cfegs in the same tissue. The appearance of seasonal Cfegs supports the recent proposal that generation of Cfegs, through eosinophil lysis, is a paradigm for the ultimate activation of airway mucosal eosinophils *in vivo* (1, 2).

Free eosinophil granules may be produced not only through inflammatory lysis mechanisms (1, 2) but also mechanically as artifacts at sampling and handling of airway tissue (and luminal material). Thus, in the present study, care was taken to obtain large tissue samples allowing the examination of mucosal sections well away from areas directly

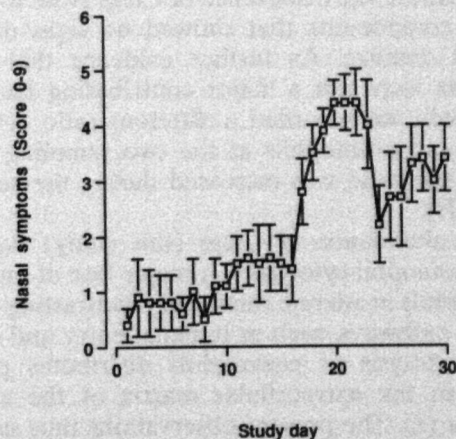


Fig. 1. Nasal symptoms scored by patients ($n=7$) during study period. Scores demonstrated significant increase in nasal symptoms during pollen season.

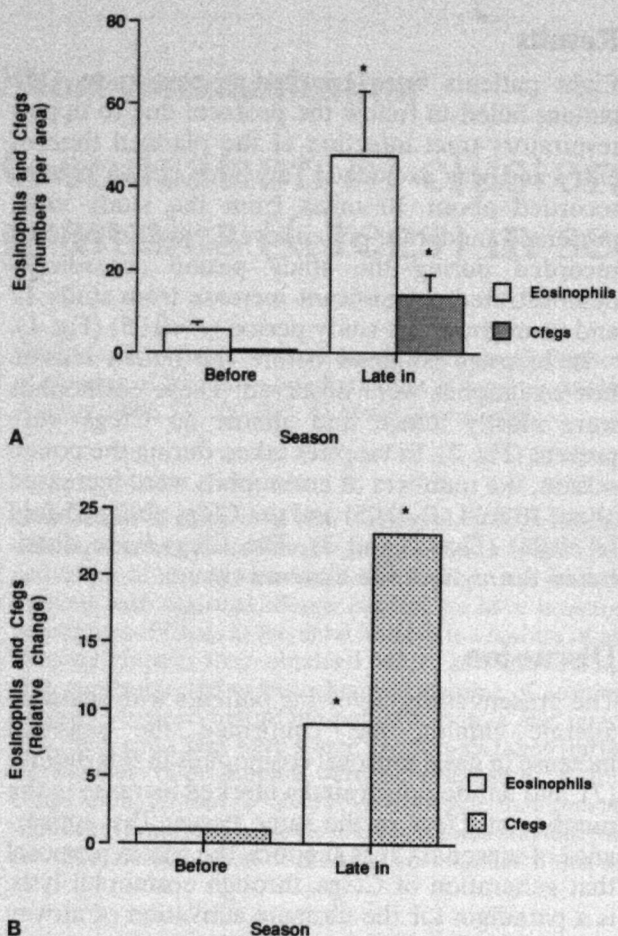


Fig. 2. Numbers of eosinophils and Cfegs per defined area (A) and relative change (B) (numbers before season set to 1) in nasal biopsies obtained from patients ($n=7$) with seasonal allergic rhinitis before and during pollen season. There was seasonal increase in numbers of eosinophils as well as Cfegs, but relative increase in Cfegs was greater ($*P<0.05$).

damaged by the punch biopsy. We could also demonstrate the occurrence of Cfegs close to other tissue components that showed no signs of artifactual damage. As further evidence that gross artifacts were not a major contributing factor in this study, we recorded a different ratio between Cfegs and eosinophils at the two sampling occasions (the ratio was increased during the season) (Fig. 2B).

The abundance of Cfegs (this study) suggests that eosinophil cytolysis is a major fate of mucosal eosinophils in allergic rhinitis. By contrasting elimination pathways, such as luminal entry and apoptosis, cytolysis of eosinophils distributes potent Cfegs in the extracellular matrix of the airway mucosa (1). The present observations thus suggest that the number of Cfegs is an important index of eosinophil activity, supplementing other staining techniques that merely identify the presence of

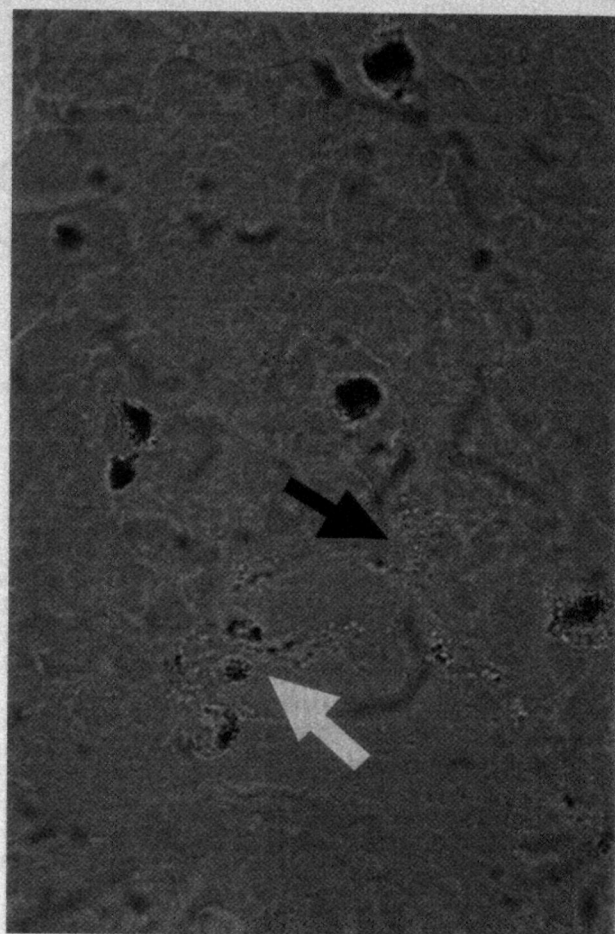


Fig. 3. Light micrograph demonstrating histochemical visualization of cyanide-resistant eosinophil peroxidase in representative nasal mucosal biopsy obtained late in pollen season (study days 27 and 28). Scattered Cfegs (indicated by arrows) were present in lamina propria among eosinophils with normal intact appearance.

these cells (including EG2 [8]). The degree to which the generation of Cfegs may correlate to other molecular, cellular, and physiologic indices and symptoms of allergic airway disease now warrants further investigation.

In conclusion this study has demonstrated a 25-fold increase in nasal mucosal Cfegs during the Swedish birch-pollen season. These data support the proposal (1, 2) that eosinophil lysis and generation of Cfegs (that leak the granule proteins in the mucosal target tissue) represent a major activation mechanism of eosinophils in allergic airway diseases *in vivo*.

Acknowledgments

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