

The increased number of epithelial mast cells in nasal polyps and adjacent turbinates is not allergy-dependent

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Respiratory epithelial mast cells are an expression of airway inflammatory processes. Nasal epithelial mast cells are known to be increased in allergic rhinitis and have now been examined in patients with nasal polyps. Metachromatic cell counts (mean $\pm$ standard error) expressed as the sum of large mast cells, atypical mast cells and basophils in epithelial scrapings of the inferior turbinates, assessed after Carnoy's fixation and toluidine blue staining (pH 0.5), were 37.5 ± 29 in non-allergic normal control subjects ($n = 11$), 435 ± 130 in polyp patients who were allergic ($n = 18$), and 699 ± 267 in polyp patients who were not allergic ($n = 8$). Metachromatic cell counts in epithelial scrapings obtained *in vivo* from nasal polyps of allergic patients ($n = 8$) were 1769 ± 962 , and 2308 ± 1544 from polyps of non-allergic patients ($n = 5$); metachromatic counts were 2089 ± 633 in epithelial scrapings from excised polyps of allergic patients ($n = 14$) and 2214 ± 640 from polyps of non-allergic patients ($n = 13$). It is concluded that the number of metachromatic cells in the epithelium of nasal polyps and the adjacent nasal mucosa is elevated compared with normal nasal epithelium and the increased number does not depend upon allergy.

Key words: epithelial mast cells; nasal polyps.

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CLINICAL ASPECTS

Increased numbers of mast cells were present in the epithelium of nasal polyps and inferior nasal turbinates of polyp patients. The large number of mast cells in the epithelium, in close relationship to inhaled air, are potentially highly responsive to inhalant factors. The number of mast cells in the nasal polyp epithelium and turbinate epithelium is not dependent upon the presence or absence of allergy. They nevertheless could still contribute to symptoms in people who are allergic and make these people highly susceptible to inhaled allergens. The nasal polyp is a good source of tissue to study airway mucosal inflammation and the findings may be representative of affected airway mucosa at other sites.

Nasal polyps can develop in allergic or non-allergic individuals. In some patients with negative allergy skin tests, evidence of local production of IgE (1) and the presence of IgE antibody (21) have been demonstrated in polyp tissue. Proposed causal factors in the development of

nasal polyps include nasal airflow obstruction (11), allergy and infection (20). Based upon histology, nasal polyps have been classified into a neutrophilic type, presumed to be due to chronic infection, and an eosinophilic type, presumed to be due to allergy (10).

An increase in metachromatic cell numbers in the respiratory tract in association with inhalant allergy has been documented for mast cells in the lung (2), nasal tissues (15) and bronchoalveolar lavage fluid (8) and for basophils in nasal secretions (23). Mast cells are present in the stroma as well as the surface epithelium of nasal polyps (4). The contribution of these metachromatic cells to the pathogenesis of the polyp is not entirely clear but degranulation suggesting an ongoing release of mediators, has been demonstrated (4, 22).

Conditioned medium from cultured polyp epithelium can stimulate the growth of basophilic and eosinophilic cells in hemopoietic colony forming assays (17). The production of colony stimulating activities by epithelial cells could explain the finding that mast cells are commonly present in polyp epithelium. An alternative mechanism would be the migration of mast cells from the lamina propria to the epithelium (5).

In allergic rhinitis (18), seasonal exposure to inhaled pollen can lead to an increase in the number of nasal epithelial mast cells. Conditioned medium from culture of allergic rhinitis nasal but not normal turbinate epithelial scrapings stimulates the growth of metachromatic cell and eosinophil-containing colonies (12). The same effect is elicited by nasal polyp epithelium from allergic or non-allergic subjects (13).

The purpose of the present study was to determine whether the number of epithelial metachromatic cells in nasal polyps and adjacent turbinates was elevated and whether the counts were allergy-dependent.

MATERIAL AND METHODS

Patients with nasal polyps were recruited from the Allergy and the ENT Clinics of McMaster University Medical Centre. None had cystic fibrosis. There were two groups of patients: one in which epithelial scrapings were taken from the polyps and the inferior turbinates *in vivo* and another whose polyps were removed surgically. No patients had received topical or systemic corticosteroids during the preceding month except where explicitly stated. The normal sub-

jects had no history of nasal disease and negative allergy skin tests with 17 common inhalant and food allergens.

Epithelial scrapings, obtained with a stainless steel curette, were taken from the inferior turbinate *in vivo*, from the surface of nasal polyps *in vivo*, or from the surface of excised polyps. The scrapings were immediately spread on a glass slide and then fixed.

Metachromatic cell counts were performed on specimens fixed for 10 min in Carnoy's solution or neutral buffered formalin and stained with toluidine blue (pH 0.5) for 5 min. The total number of metachromatic cells in each specimen was counted; these included mast cells, atypical mast cells and basophils (7).

To examine the reproducibility of the scraping method, three scraping specimens were obtained from the inferior turbinate or polyp of individual patients at the same visit. Total metachromatic cell counts were performed by an observer blinded to the origin of the slides. Mean counts ranged from 7 to 3823. The intraclass correlation co-efficient, calculated from output from a two-way analysis of variance procedure, was 85 % (16). The good reproducibility corresponds to that previously shown with this method (17).

For tissue segments, excised polyps were placed in McCoy's 5A medium and carried directly to the laboratory. Corresponding segments of tissue, containing epithelium and stroma were removed and placed in either Carnoy's solution or 10 % buffered formalin (pH 6.9) for fixation. Following preparation of paraffin sections, each section was stained with 0.5 % toluidine blue in 0.7 NHCl at pH 0.5.

The total serum IgE concentration was determined by the PRIST method (Pharmacia Canada Inc., Montreal, Canada). Skin tests were performed by the prick test method with extracts of common inhalant and food allergens (Bencard Allergy Laboratories, Mississauga, Canada). These were *Alternaria*, *Homodendrum*, *Aspergillus fumigatus*, *Candida albicans*, egg white, milk, wheat, dog epidermis, cat epidermis, horse epidermis, *D. farinae*, *D. pteronyssinus*, *Blatella germanica*, tree pollen mix, grass pollen mix, ragweed pollen and weed pollen mix.

Results were graded from 0 to 4+. A skin test index is used consisting of the number resulting from the addition of pluses in a skin test set. Patients were defined as non-allergic if all skin tests were negative.

Results are expressed as the mean and the standard error of the mean. Statistical analyses were performed with the Mini-Tab Statistics Program. Statistical significance was determined using two-tailed unpaired Student's t test unless otherwise stated. Correlation coefficients were determined by linear regression analysis.

RESULTS

Cells with basophil morphology constituted a small proportion of the total metachromatic cell counts. The percentage of the metachromatic cells that had basophil morphology were, in turbinate scrapings ($n = 17$) range = 0-24, mean 5.0, median = 3.0, and in polyp scrapings, ($n = 23$), range 0-17, mean 4.7, median 3.0. Separate consideration of the metachromatic cells with or without the basophil counts did not change the statistical significance of the findings and results below are therefore expressed as total metachromatic cell counts.

Epithelial scrapings from surfaces of 21 polyps were fixed in Carnoy's solution followed

by toluidine blue stain, and compared with scrapings from the same polyps fixed in formalin followed by toluidine blue stain (11 patients had been on corticosteroid treatment). Metachromatic cell counts of specimens fixed in Carnoy's were 2291 ± 448 and in formalin were 82 ± 46 (paaired t test, $P < 0.0001$). Accordingly, all subsequent counts are reported for Carnoy's fixation.

Metachromatic cell numbers were also counted in the stroma of sections from nine polyps (one patient had received steroid in the preceding month). Counts of Carnoy's fixed sections were 156 ± 28 and 107 ± 23 in formalin fixed sections.

Scrapings from the inferior turbinate of patients with nasal polyps ($n = 26$) had 516 ± 121 metachromatic cells compared with normal subjects ($n = 11$) with 38 ± 29 ($P = 0.0007$). For allergic patients with polyps ($n = 18$), the counts were 435 ± 130 (compared with normal, $P = 0.008$), and for non-allergic patients ($n = 8$) with polyps were 699 ± 267 (compared with normals, $P = 0.043$) Fig. 1).

Counts for *in vivo* epithelial scrapings of polyps from allergic patients ($n = 8$) were 1769 ± 962 and from non-allergic patients ($n = 5$) were 2308 ± 1544 ($P = 0.78$). Counts of metachromatic cells in scrapings from excised polyps from patients with allergy ($n = 14$) were 2089 ± 633 and without allergy ($n = 13$) were 2214 ± 640 ($P = 0.89$) (Fig. 2).

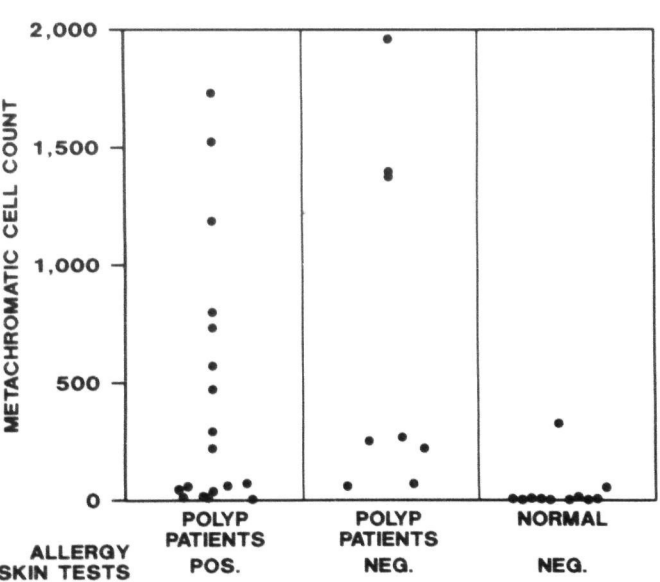


Fig. 1. Nasal turbinate epithelial scrapings.

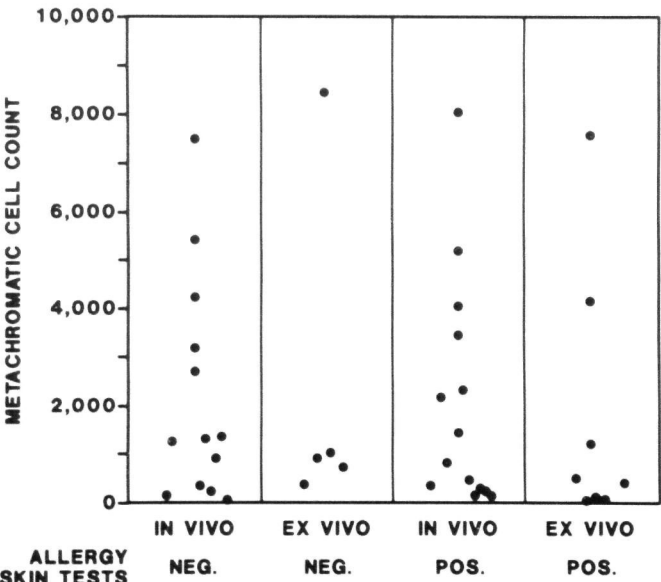


Fig. 2. Nasal polyp epithelial scrapings.

Table 1
Relationship between mast cell counts and allergy*

Source of epithelial scrapings	No. of cases	Correlation coefficient
Nasal polyp		
metachromatic cell count vs skin test index	39	-0.06
metachromatic cell count vs serum IgE	37	-0.166
Inferior turbinate of polyp patients		
metachromatic cell count vs skin test index	13	-0.243
metachromatic cell count vs serum IgE	13	-0.006

*None of the patients had received topical nasal or systemic steroid therapy during the preceding month.

Correlations between the number of metachromatic cells in the epithelium of nasal turbinates or of nasal polyps and the allergy skin test indices or the serum IgE concentrations of the patients with nasal polyps are shown in Table 1. The metachromatic cell counts did not correlate positively with the presence of allergy, determined by allergy skin tests or serum IgE concentrations.

DISCUSSION

The observed increase in the number of mast cells in the nasal turbinate epithelium of patients with polyps (16, 19) is indicative of ongoing inflammation in these tissues. Inflammation, generally represented by mast cells and eosinophils, is also characteristic of the stroma of nasal polyps (4). The lack of a relationship between the epithelial mast cell numbers and the allergic status of the patients, whether assessed by skin test reactivity or by serum IgE concentration, illustrates that mechanisms other than allergy are involved in the accumulation of cells in the inflammatory process.

In general, in IgE-dependent reactions, an influx of eosinophils can occur within 3 h after antigen challenge; this could be explained primarily by a transfer from the blood in response to a chemotactic stimulus (1). There is no knowledge of a corresponding rapid appearance of mast cells. Previous observations provide evidence of

in situ hemopoetic mechanisms as a basis for local inflammatory cell accumulation (17). The observation that progenitor cells from polyp tissue have a unique tendency to differentiate towards basophilic cells rather than eosinophils (17) is of particular interest in this context.

The metachromatic staining of Carnoy's and formalin-fixed polyp epithelial scrapings and polyp sections illustrates a disparity between the mast cell populations in the two separate locations. Most polyp stroma mast cells were formalin resistant while almost all epithelial scraping mast cells were formalin sensitive, with demonstrable metachromatic staining only after Carnoy's fixation. Such phenotypic differences are usually indicative of functional differences (6, 8).

The etiology of polyps remains unknown. The observed increase in turbinate epithelial mast cell numbers and the high counts in the polyp epithelium suggests that comparable processes are operative. This is not surprising in a condition where there is often coexisting polyposis, sinusitis, rhinitis and asthma. There is increasing evidence of the importance of a variety of cytokines, such as GM-CSF, in the lineage-specific development of inflammatory cells (7, 14). We have previously demonstrated the production of colony stimulating activities by polyp epithelium (8, 14, 17). Recently, we have observed a multiplicity of effects upon cellular differentiation from nasal polyp and nasal turbinate fibroblast and epithelial monolayers and their conditioned media in studies with the HL60 leukemic cell line (7). Cytokines such as GM-CSF, IL-3, IL-5 and nerve growth factor (9) have effects on basophil/mast cell differentiation and some of these may be responsible for the presence of metachromatic cells and eosinophils within nasal polyps.

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