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Mast cell numbers in the mucosa of the inferior turbinate in patients with perennial allergic rhinitis: a light microscopic study

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Abstract
The number of mast cells in the inferior turbinate from patients with perennial allergy due to house dust mite were compared with ten normal controls. Ten random high powered fields were counted in the epithelium and the submucosa in samples which had been divided into two and fixed either in aqueous formalin or Carnoy's fixative. The sections from each block were stained with either Azure A or Chloroacetate esterase technique. No statistical differences were found. The lack of increase in mast cell numbers was attributed to degranulation since numbers have been shown to be increased in perennial allergy when sections are examined ultrastructurally.

Introduction

Mast cells are widely distributed through the nasal mucosa and are most dense just beneath the surface epithelium in the normal nose (Trotter *et al.*, 1990). The numbers increase in seasonal allergic rhinitis. Cells may migrate into the surface epithelium which may explain the priming that occurs in this condition (Enerback *et al.*, 1986; Viegas *et al.*, 1987). There is little data available on patients with perennial allergic rhinitis where many of the patients have continuous allergen exposure unlike seasonal allergic rhinitis where there is marked variations from day to day and time of day. All phases of the allergic response may be present and mast cells numbers should be increased all the time.

The numbers of tissue mast cells do not appear to be raised in patients with nasal polyps where an increase in tissue histamine levels is found and mast cells are degranulated (Cauna *et al.*, 1972; Drake-Lee and McLaughlin, 1982). Similarly, they are not present in increased numbers in the surface epithelium (Drake-Lee *et al.*, 1988).

One of the problems in studying mast cell numbers with the light microscope is the methods by which they are fixed and stained. Some mast cells are sensitive to formol fixation and do not stain well so that alcohol based fixatives may give better results. Different stains may demonstrate mast cells better by using various techniques, namely, metachromasia and intracellular enzymes (Enerback *et al.*, 1986; Viegas *et al.* 1987).

The aims of this study were to look at the distribution and the numbers of mast cells in the inferior turbinate both in patients with normal nasal mucosa and in patients with perennial allergic rhinits. Tissue was fixed in both formol and alcohol solutions and stained with azure A and by the chloroacetate esterase method.

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Materials and methods

Normal subjects

Ten patients who were admitted for septoplasty or septorhinoplasty were studied. All patients had no history of nasal disease apart from trauma and no symptoms apart from unilateral nasal obstruction. They all had negative skin tests and the biopsies were taken from the middle of the inferior turbinate on the unobstructed side.

Patients with perennial allergic rhinitis

Ten patients were studied who had a history of nasal symptoms on exposure, inparticular, nasal blockage and had positive skin tests to two or more allergens including house dust mite extract using the prick method (Bencard). Patients were admitted for turbinate surgery since medication had failed to control their symptoms and no current medication had been taken for one month prior to their admission. All biopsies were taken from the middle of the inferior turbinate.

Nasal preparation

Patients had preoperative preparation of the nose with cocaine solution (10 per cent) prior to surgery and further preparation with cocaine paste (25 per cent) in the anaesthetic room. All biopsies were divided into two with one sample being placed into formol saline solution and the other into Carnoy's solution (chloroform, ethanol and acetic acid).

Histological preparation

Tissues were processed in the usual way and embed-

ded in paraffin wax. Sections of 3–5 m were cut and stained with azure A and by the chloroacetate esterase method (Hopwood, 1982).

Light microscopy

All metachromatic cells in ten high powered field both in the epithelium and the connective tissue were counted.

Statistical evaluation

Results were compared by the Mann-Whitney rank test.

Results

The data are summarized in the table and show that the results are consistent with the Null hypothesis that there is no differences between the two fixation methods.

Very few mast cells were demonstrated in the epithelium with either fixative or staining technique. The majority of the mast cells were in the connective tissues and the numbers were slightly higher when Carnoy's fixative and the choroacetate esterase method was used. The only trend in the results was that the counts were higher in the submucosa in allergic patients if formol saline fixative was used and the tissues were stained with azure A but this did not reach statistical significance.

Discussion

Although the allergic reaction is centred on mast cell degranulation, there is still considerable debate on the best way to study mast cells. Mast cells contain granules which may be demonstrated by a number of ways. The cell binds IgE onto its surface avidly but the granules disappear by degranulation.

Mast cells were first identified by Ehrlich when he was a medical student (Ehrlich, 1879) and the sensitivity of mast cells to fixation was described initially by Hardy and Westbrook (1895) who investigated the fixation properties of mast cells in a number of animals excluding man. This property has been developed more recently by Ennerback and his colleagues who hoped to demonstrate similar properties in human with the anticipation of classifying human mast cells into connective tissue and mucosal mast cells (Enerback, 1981). It has been suggested that such a classification is possible in the human gastrintestinal tract (Strobel *et al.*, 1981).

TABLE I

	Epithelium			
	Formol		Carnoy's	
	Azure A	CAE	Azure A	CAE
Normal	0 (0–2)	0 (0–9)	0 (0–2)	0 (0–5)
Allergic	0 (0–3)	1 (0–3)	0 (0–6)	0 (0–9)
	Connective tissue			
Normal	7 (1–26]	12 (0–19)	5 (0–23)	10 (0–31)
Allergic	12 (1–17)	12 (3–24)	5 (0–17)	12 (0–26)

The values are the median and range of cell numbers found in the inferior turbinates of normal controls and patients with perennial allergic rhinitis when fixed in either formal solution or Carnoy's fixative and stained either with Azure A or by the chloroacetate esterase (CAE) method.

Fixation is a variable property which may be reversible with formalin particularly when longer periods are used. Carnoy's fixative damages tissues but does preserve nucleases better than routine fixatives. (Michels, 1938; Hopwood, 1982). The variable results from fixation make studies of mast cells difficult and so most authors use both methods to demonstrate mast cells to try and distinguish mast cell populations.

While the rat does have two distinct populations of mast cells, connective tissues and mucosal types, the evidence for this in man is less certain. The problems of demonstrating and classifying mast cells have been discussed fully elsewhere (Drake-Lee and Price, 1990a).

Patients with seasonal allergic rhinitis have an increase in mast cell numbers in the nose which also includes the epithelium during the time of allergen challenge. The numbers in the epithelium drop out of season (Enerback *et al.*, 1986; Viegas *et al.*, 1987). Patients with nasal polyps, which in most cases is not an allergic condition, have no evidence of an increase in mast cell numbers either in the epithelium or in the connective tissue both in the polyp itself and the inferior turbinate (Drake-Lee *et al.*, 1988). Drake-Lee and his colleagues confirmed that mast cells were essentially found below the epithelium and used tissue fixed in both formol and Carnoy's solutions. They concluded that although Cornoy's fixative did demonstrate mast cells slightly better, there was no obvious subclassification possible on site, fixation and staining and, also, that the classification in rats might be an over-simplication when applied to man.

Chloroacetate esterase is not specific for mast cells and stains polymorph leukocytes as well. Previously, we have not found that altering the staining time makes much difference on the types of cells demonstrated. These results confirm the previous study concerning the distribution and staining of mast cells. Unlike the prevous studies on patients with seasonal allergic rhinitis we could not demonstrate an increase in mast cell numbers (Enerback *et al.*, 1986; Viegas *et al.*, 1987). We have found that patients with perennial allergic rhinitis due to house dust mite allergy do have increased numbers of mast cells when tissue is examined under the electron microscopy but many of the cells have very little material in the granules and that only a ghost of a scroll or other electron dense material is present in the granule (Drake-Lee, Price 1990b). The failure to demonstrate an increase in mast cell numbers here may then be due to a failure to demonstrate degranulated cell. The absence of an increase in mast cells in the surface epithelium suggests that the reactions are submucosal.

In conclusion, we cannot demonstrate marked differences in mast cell numbers in patients with perennial allergic rhinitis due to dust mite sensitivity when compared with controls. We were also unable to show that different fixation and staining improved the yield of mast cells.

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