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Increase in epithelial mast cell numbers in the nasal mucosa of patients with perennial allergic rhinitis

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Abstract

The aim of the study was to compare the numbers and distribution of mast cells in the nasal mucosa of perennial allergic rhinitis (PAR) patients and controls, as demonstrated by different staining methods for light microscopy.

Biopsies of inferior turbinate mucosa were taken from 10 patients with PAR and 10 patients undergoing septoplasty or septorhinoplasty (control group). Sections for light microscopy were stained with azure A, chloroacetate esterase and an ABC immunohistochemical technique using antibody to tryptase.

Three times more mast cells were found in the epithelium of PAR patients compared to controls using the immunohistochemical technique ($p = 0.0074$). This method demonstrated considerably more mast cells than the other stains.

The increase in epithelial mast cells is consistent with the migration of mast cells seen in seasonal allergic rhinitis, and this may be important in the phenomenon of nasal priming seen after repeated antigen exposure.

Key words: Immunohistochemistry; Mast cells; Rhinitis, allergic, perennial

Introduction

Mast cells are commonly found in the nasal mucosa and are thought to have a central role in allergic disease. Mast cells avidly bind IgE to their surface and antigen cross-links these molecules to trigger degranulation. The mediators of allergic disease derived from the mast cell cytoplasmic granules include histamine, heparin, eosinophil and neutrophil chemotactic factors, prostaglandin D_2, leukotriene C_4 and neutral proteases such as tryptase. These have direct actions themselves and cause the release of other mediators, especially the products of arachidonic acid metabolism, by different cells (Peters *et al.*, 1987; Schwartz *et al.*, 1987).

Mast cells are the only cells known to contain tryptase, and recently a murine monoclonal antibody against tryptase has become available commercially (Walls *et al.*, 1990a; Walls *et al.*, 1990b). Immunostaining with anti-tryptase has been reported as being a very sensitive and specific method of detecting mast cells (Craig *et al.*, 1986; Irani *et al.*, 1986; Irani *et al.*, 1989; Morgan and William, 1991). Prior to this, mast cells have been demonstrated by use of metachromatic dyes such as azure A and toluidine blue, or by the chloroacetate esterase method, an enzymatic stain that makes use of esterases in the mast cell cytoplasm. Neither of these are entirely specific for mast cells. Metachromasia is also shown by basophils (Cook, 1982) and esterases are also found in neutrophil polymorphs (Li *et al.*, 1973). A problem with the demonstration of mast cells by light microscopy is that degranulation reduces the content of the cytoplasmic mediators responsible for the staining properties (Fokkens *et al.*, 1992).

An increase in epithelial mast cell numbers has been seen in seasonal allergic rhinitis (SAR) patients during the pollen season compared to out of season or controls (Enerback *et al.*, 1986; Viegas *et al.*, 1987; Bentley *et al.*, 1992). This has not been shown in PAR (Drake-Lee *et al* 1991). Although the same underlying process is thought to be responsible for both diseases, the different patterns of allergen exposure result in different symptom spectrums and may show different cellular responses (Kaliner, 1992).

The aims of this study were to compare the numbers and distribution of mast cells in the nasal mucosa of PAR patients and controls, as demonstrated by azure A, chloroacetate esterase and immunostaining using anti-tryptase antibody.

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TABLE I

MEAN NUMBER OF CELLS FOUND IN TEN HIGH POWER FIELDS OF EPITHELIUM AND CONNECTIVE TISSUE FOR DIFFERENT STAINING AND FIXATION METHODS. FIGURES IN PARENTHESES ARE THE RANGE OF CELL NUMBERS

Epithelium fixed with formal saline	azure A	esterase	immuno-
control	0.8 (0–5)	0.4 (0–3)	4.7 (0–19)
allergic	1.6 (0–10)	0.6 (0–4)	13.0 (1–43)
Epithelium fixed with Carnoy's fixative	**azure A**	**esterase**	**immuno-**
control	0.2 (0–1)	0.1 (0–1)	4.6 (0–16)
allergic	0.4 (0–2)	0.6 (0–3)	12.6 (0–28)
Connective tissue fixed with formal saline	**azure A**	**esterase**	**immuno-**
control	4.2 (0–20)	2.9 (0–14)	20.3 (3–42)
allergic	2.7 (0–12)	2.9 (0–8)	17.2 (0–42)
Connective tissue fixed with Carnoy's fixative	**azure A**	**esterase**	**immuno-**
control	2.8 (0–15)	2.6 (0–7)	20.5 (5–41)
allergic	1.1 (0–8)	1.3 (0–5)	18.4 (0–55)

Methods and materials

Patients were diagnosed with PAR on the basis of a history of nasal symptoms upon exposure to house dust and positive skin tests to house dust or house dust mite extract using the skin prick method. They had been admitted for turbinate surgery since medication had failed to control their symptoms. No medication had been taken for one month prior to admission. All biopsies were taken from the middle of the inferior turbinate.

Control biopsies were obtained from patients admitted for septoplasty or septorhinoplasty. They had no history of nasal disease apart from unilateral nasal obstruction and had negative skin tests to a range of common allergens. All biopsies were taken from the middle of the inferior turbinate of the unobstructed side.

Biopsies were divided into two, one half being fixed in 10 per cent formal saline, the other in Carnoy's fixative. Carnoy's fixative is absolute ethanol 60 per cent, chloroform processed routinely for light microscopy, while tissue fixed in Carnoy's fixative was processed using absolute ethyl alcohol. All specimens were blocked into paraffin wax. Sections were cut at 4 μm thickness and stained with azure A (Cook, 1982), chloroacetate esterase (Li *et al.*, 1973) and anti-tryptase (Dako) ABC immunoperoxidase method (Walls *et al.*, 1990b). Anti-tryptase antibody was used at a dilution of 1 in 2000. Sections fixed in formal saline required pre-treatment with trypsin before immunostaining. This was not necessary for tissue fixed in Carnoy's fixative. In addition, one formal saline-fixed section and one Carnoy's fixed section were used as controls for immunoperoxidase staining by omitting the primary anitbody.

TABLE II

MEAN NUMBER OF CELLS PER MM^2 FOR DIFFERENT STAINING AND FIXATION METHODS

Connective tissue fixed with formal saline	azure A	esterase	immuno-
Control	7.8	5.4	37.6
Allergic	4.9	5.3	31.8
Connective tissue fixed with Carnoy's fixative	**azure A**	**esterase**	**immuno-**
Control	5.1	4.7	37.9
Allergic	2.0	2.5	34.0

Sections were examined by light microscopy and the numbers of positively-staining cells in 10 high-power fields of epithelium and 10 high power fields of connective tissue were counted. In sections stained using the chloroacetate esterase technique, positively staining cells with polymorphic nuclei were judged to be neutrophil polymorphs and were not counted. Quantitative values of mast cells per mm^2 were calculated for connective tissue fields. This could not be done for the epithelial tissue since epithelium did not fill an entire high power field. Thus, epithelial cell counting did not compare identical areas of epithelium, but identical lengths. Epithelial thickness was not accounted for.

Epithelial cell counting was repeated by the original observer and a different observer.

Results

Biopsies from 10 PAR patients and 10 controls were processed for light microscopy. Control immunoperoxidase sections showed no positive staining. The results of the cell counting are shown in the tables. Table I shows the mean number of cells and range for all results. Table II shows the mean number of cells per mm^2 for cell counting in the connective tissue only. Table III shows the statistical significance for cell counting of immunostained epithelial tissue.

Immunostaining of Carnoy's-fixed tissue demonstrated a significantly greater number of mast cells in the allergic group compared to the control group ($p = 0.0433$ Mann–Whitney test). This was not so for immunostaining of formal saline fixed tissue, but when the numbers of cells in tissue fixed by both methods was totalled, there was also found to be significantly more in the allergic group than the

TABLE III

SIGNIFICANCE VALUES FOR CELL COUNTS OF IMMUNOSTAINED EPITHELIAL TISSUE

Control	Formal saline	4.7	(0–19)	NS		
	Carnoy's fixative	4.6	(1–43)		$p = 0.0433$	
Allergic	Formal saline	13.0	(0–16)			$p = 0.0074$
	Carnoy's fixative	12.6	(0–28)			

Table shows mean number of cells found in ten high power fields of epithelium, for immunostaining and different fixation methods. Figures after the brackets refer to statistical significance. ($p = 0.0433$ Mann–Whitney test)

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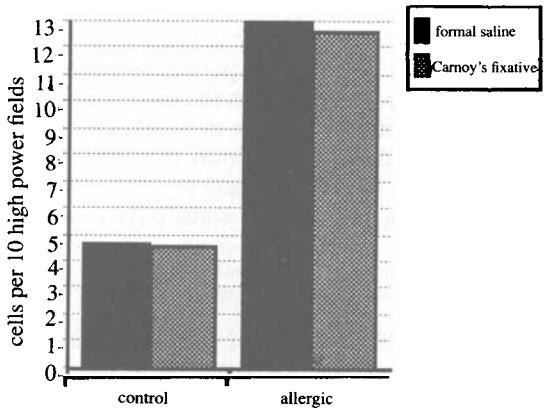

FIG. 1

Mean numbers of mast cells per 10 high power fields of epithelium as demonstrated by immunostaining

control group ($p = 0.0074$, Mann-Whitney test) (Figure 1).

There was no significant difference between number of cells demonstrated using the azure A and chloroacetate esterase techniques, but immunostaining showed more cells than azure A and than chloroacetate esterase (both $p<0.001$, t-Test). In no case were there significantly more cells demonstrated on Carnoy's-fixed tissue than formal saline-fixed tissue.

Epithelial cell counting was repeated by the same observer and by a different observer. The repeatability coefficients for both intra- and interobserver variation were within acceptable limits as described by Bland and Altman (1986).

Discussion

These results show that there was no significant difference between mast cell numbers demonstrated by the azure A and chloroacetate esterase techniques, and that immunostaining demonstrated considerably more cells than these two methods. Walls *et al.* (1990b) reported the use of anti-tryptase on various tissues, but not nasal mucosa. They compared immunostaining with adjacent serial sections stained with toluidine blue, a metachromatic dye similar to azure A, and showed identical staining patterns. However, immunostaining did demonstrate significantly more cells, and this has also been seen by Morgan and William (1991) in conjunctiva and by Craig *et al.* (1986) in lung and small intestine.

Degranulation of mast cells reduces the content of the mediators responsible for staining. The great sensitivity of anti-tryptase immunostaining may enable detection of extensively degranulated cells that would not be seen by other methods, resulting in the demonstration of more cells. This could be tested by double immunostaining with anti-IgE and anti-tryptase, or by immunogold electron microscopy using anti-tryptase to determine if extensively degranulated cells retain some anti-tryptase staining properties. Igarashi *et al.* (1993) studied normal mucosa with double immunostaining with anti-IgE and anti-tryptase and showed that almost all IgE positive cells also stained for tryptase and therefore were mast cells. Conversely, a third of mast cells were IgE-positive, as also found by Fokkens *et al.* (1992) who used anti-IgE and toluidine blue staining. In addition, they showed that in allergic mucosa, all mast cells were IgE positive.

Heterogeneity of mast cells was first demonstrated by the different sensitivity to fixative type seen when metachromatic staining was used. Fixation with formaldehyde blocks the polyionic areas on proteoglycans responsible for metachromatic staining and so reduces the intensity of staining. An atypical type of mast cell has been described, possessing a distinctive type of proteoglycan in its granules which is more sensitive to formaldehyde blockage (Pipkorn *et al.*, 1988), and so fewer of these cells are demonstrated when formaldehyde is used as the fixative than when alcohol-based fixatives such as Carnoy's are used (Strobel *et al.*, 1981; Otsuka *et al.*, 1985; Barrett and Metcalfe, 1987; Kitamura, 1989; Drake-Lee and Price, 1992; Pesci *et al.*, 1993). This distinction may be clinically important because similar subsets of mast cells isolated from rats show different sensitivity to anti-degranulation agents such as chromoglycate *in vitro* (Pearce *et al.*, 1982).

No evidence was found in this study for heterogeneity of mast cells in the nasal mucosa based upon sensitivity to fixation. Otsuka *et al.*, (1985) showed that mast cells present in nasal scrapings (mostly epithelial cells, but also secreted cells) were over 80 per cent atypical. Drake-Lee *et al* (1991) used biopsies and found that fixative type had no effect upon numbers of cells demonstrated in either the epithelium or in connective tissue. A recent study by Kleinjana *et al.* (personal communication) comparing fixation with Carnoy's fluid to acetone fixation has shown that Carnoy's fluid reduces the number of chymase-positive mast cells, but only reduces tryptase-positive mast cells after staining for 10 minutes. This indicates that acetone fixation produces superior results to fixation with Carnoy's. All mast cells contain tryptase, and only a proportion contain chymase, and the difference in staining results using chymase or tryptase is probably a result of the antibodies used in the immunostaining technique, rather than reflecting mast cell heterogeneity.

Immunostaining with anti-tryptase showed that there were approximately three times the number of mast cells in the epithelium of PAR patients compared to controls. The histochemical methods failed to show this difference. Most authors agree that there is an increase in epithelial mast cell numbers in antigen-challenged SAR patients (Enerback *et al.*, 1986; Viegas *et al.*, 1987; Bentley *et al.*, 1992; Fokkens *et al.*, 1992), and that this is due to migration (Fokkens *et al.*, 1992).

The results of this study are consistent with migration seen in SAR, and this has never been seen in PAR before. Okuda *et al.* (1983) have shown an increase in basophilic cells in scrapings of nasal mucosa from PAR patients compared to controls. Scrapings take cells free in the nasal secretions as well as epithelial tissue and are an inconsistent

sampling method. Basophils are the commonest cells in nasal secretions (Otsuka *et al.*, 1985) and the staining method used did not distinguish between mast cells and basophils. Okuda *et al.*, (1983) also studied basophilic cells in the lamina propria using biopsies, and found no difference in numbers compared to controls. Drake-Lee *et al.*, (1991) found no difference in mast cell numbers in PAR patients and controls in either the connective tissue or epithelium using azure A and chloroacetate esterase staining, and formaldehyede and Carnoy's fixation.

Since identical lengths and not areas of epithelium were compared, it could be possible that the increased numbers of mast cells wre due to thickening of the epithelium in the PAR patients. Although this was not quantified, epithelial thickness did not appear to be significantly different between biopsies. Work by Otsukai *et al.*, (1993) using biopsies from PAR patients has shown that numbers of epithelial mast cells do not increase in relation to epithelial thickness.

Since the degree of convolution of the surface of the mucosa would result in variable lengths being contained in one high power field, this could affect the results of the cell counting. However, in all biopsies the epithelium was rarely seen to be anything other than flat, and this factor is unlikely to have affected the results, although it was not quantified.

As the pollen season progresses the numbers of pollen grains needed to elicit symptoms in SAR patients is reduced, a phenomenon known as priming. This is thought to be due to increased production of IgE, but may also be due to migration of mast cells into the epithelium, which is more accessible to antigen.

Several observations reviewed by Kawabori *et al.* (1983) suggest that the reactions of cells in the epithelium and nasal secretions are of prime importance in the immediate reaction. Topical corticosteroids are effective in reducing symptoms in allergic rhinitis, especially the late phase and the priming response (Pipkorn *et al.*, 1987b; Bascom *et al.*, 1988; Juliusson *et al.*, 1993). Evidence about the effects of corticosteroids upon mast cell numbers are conflicting, probably due to the problems of demonstrating degranulating mast cells and of standardising antigen exposure (Pipkorn and Enerback, 1987a; Gomez *et al.*, 1988; Juliusson *et al.*, 1993). Despite this, it seems likely that corticosteroid treatment prevents an increase in mast cell numbers in response to allergen challenge, and this may be related to their action in reducing the priming effect. Mast cell precursors are probably recruited into the epithelium by the action of cytokines released from degranulating mature mast cells or T lymphocytes, and corticosteroids will reduce cytokine production, and so inhibit migration.

Conclusion

Immunostaining using anti-tryptase is superior to metachromatic or enzyme staining for the demonstration of nasal mast cells. Using this method, a threefold increase in epithelial mast cell numbers was demonstrated in PAR patients compared to controls. This is consistent with the migration of mast cells into the epithelium seen in SAR, and may be responsible for the phenomenon of nasal priming. No evidence was found for mast cell heterogeneity in the nasal mucosa based upon sensitivity to fixation.

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