

Evidence for an inflammatory pathophysiology in idiopathic rhinitis

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Summary

Background The pathophysiology of idiopathic rhinitis is unknown but the disease is classified as being non-allergic on the basis of negative serum IgE radioallergosorbent assay (RAST) and skin prick tests. In contrast, allergic rhinitis has a Th2 type inflammatory pathology mediated by IgE and mast cells.

Objective To test the null hypothesis that there would be no difference in the cellular infiltrate for key Th2-associated inflammatory cells between allergic and idiopathic rhinitis.

Methods We applied strict selection criteria in the recruitment of allergic and idiopathic rhinitis cases. In contrast to previous studies which used cytology or small biopsies, we studied all layers of the mucosa by using whole, full-thickness nasal turbinate specimens with an average length of 2.5 cm. Immunohistochemistry and *in situ* hybridization techniques were used to compare the distribution and cell populations of mast cells, IgE positive (IgE⁺) cells, eosinophils and plasma cells in perennial allergic ($n = 11$) and idiopathic ($n = 17$) rhinitis, and control nasal mucosal tissue ($n = 9$).

Results Mast cells and IgE⁺ cells were significantly increased within the epithelium of allergic and idiopathic mucosa compared to normal mucosa ($P < 0.05$). More IgE⁺ cells were present in the allergic group compared to the idiopathic group with the majority of IgE⁺ cells being mast cells. Both rhinitic groups showed increased eosinophilia localized to the superficial submucosa compared to normal mucosa ($P < 0.05$). More plasma cells were present in the allergic rhinitic tissue.

Conclusion Idiopathic and allergic rhinitic mucosa show similarities in their inflammatory infiltrate suggesting that both groups share a highly localized Th2, IgE-mediated cellular immunopathology.

Keywords: Allergic rhinitis, idiopathic rhinitis, inflammatory cells, Th2 cells

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Introduction

Rhinitis affects 20% of the western population [1] with 28–60% of these having 'idiopathic' rhinitis (IR) [2] which is defined as rhinitis with no proven immunological, microbiological, pharmacological, hormonal or occupational cause [3–5]. In contrast, allergic rhinitics have a Th2 inflammatory

pathophysiology involving a series of interacting inflammatory and associated mucosal cells. The process is tightly regulated by Th2-type cytokines [6] and their receptors [7,8]. Mast cells [9–11], IgE positive (IgE⁺) cells [12], CD25⁺ (activated T cells) [13,14], and eosinophils [14] are increased in allergen-induced rhinitis, but in contrast, there have been few studies quantifying Th2-associated inflammatory cells in idiopathic rhinitis [15,16].

Previous studies of rhinitis have examined mucosal inflammatory infiltrates with sampling techniques including

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nasal brushings and scrapings [12,17], nasal washings [8,18,19] or small mucosal biopsies [9,10,13,14], allowing only epithelial or superficial mucosal cells to be investigated. To ensure we investigated inflammatory cells which are present in the deeper mucosa [15], we studied full-thickness mucosa, similar to Berger *et al.* (1997) [16]. We collected a bank of tissue comprising perennial allergic rhinitis, idiopathic rhinitis and normal nasal mucosa obtained from rigorously screened patients seen at a specialist nasal clinic. Orientated full-length, full-thickness inferior turbinate mucosa was studied using immunohistochemistry and *in situ* hybridization to determine the distribution and numbers of mast cells, IgE⁺ cells, eosinophils and plasma cells. We tested the null hypothesis that there would be no difference in the cellular inflammatory infiltrate in idiopathic rhinitis-derived mucosa compared to allergic rhinitic tissue.

Materials and methods

Selection of patient groups

Turbinate mucosa was collected from three patient groups according to strict selection criteria (Table 1). We excluded patients with autonomic rhinitis ('vasomotor rhinitis') whose primary symptom was clear rhinorrhoea with minimal symptoms of nasal obstruction, who responded to ipratropium bromide.

The first group had perennial allergic rhinitis ($n = 11$; mean age 25.7 years), the second had idiopathic rhinitis ($n = 17$; mean age 34.6 years), and the third comprised a control group without any evidence of rhinitis ($n = 9$; mean age 24.9 years). The latter group comprised non-atopic, non-allergic patients undergoing surgery for nasal obstruction primarily resulting from an injury to the nose. Patients were selected from those free of rhinitic disease before and after their nasal surgery. At endoscopy, none of the patients had purulent disease or polyposis. There were no distinguishing endoscopic features to separate the perennial allergic and idiopathic groups. Minor mucosal oedema was seen in both groups and all patients had normal CT scans. Patients with a history of asthma or eczema were excluded.

Seasonal allergic rhinitis were excluded. All the rhinitic patients were symptomatic at the time of surgery. The perennial allergic patients had presented with more than one positive skin prick test and raised specific IgE titres. The rhinitic groups underwent surgery for obstruction with turbinectomies being performed to help alleviate their symptoms.

Immunohistochemistry

Ethical approval and the patients consent were obtained prior to turbinectomies being performed. Inferior turbinate

Table 1. The following criteria were used in the selection of Idiopathic Rhinitic patients

Inclusion criteria

- Age between 16 and 64
- Negative skin prick test and ELISA (DPC AlaStat, USA) to house dust mite, other perennial allergens including dog, cat, and moulds mix
- Symptoms for more than 1 years
- A minimum of 2 out of 3 of the following symptoms: periods of nasal discharge, sneezing and nasal obstruction for an average of at least 1 h per day for at least 5 days
- Endoscopy shows no purulent disease

Exclusion criteria

- Clear rhinorrhoea and response to ipratropium bromide (excludes Vasomotor Rhinitis from our group)
- The use of systemic or nasal corticosteroids within the past 6 weeks
- Use of nasal sodium cromoglycate or nedocromil sodium within the last 6 weeks
- Use of systemic or nasal antihistamines within the previous month
- Abnormal sinus CT scan
- Purulent discharge at nasal endoscopy
- History of asthma or eczema
- Pregnancy or lactation
- Abnormal laboratory test results for: blood-sodium, potassium, calcium, total protein, albumin, urea, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, gammaglutamyl transpeptidase, haemoglobin, red blood cell count, plasma cell volume, mean corpuscular volume, platelets, total white blood cell count, neutrophils, lymphocytes, monocytes, eosinophils, basophils. Urine-blood, protein, glucose.
- Presence of raised angiotensin converting enzyme and anti-neutrophil cytoplasmic antibody.

specimens were surgically removed under general anaesthesia without vasoconstriction in the Department of Otorhinolaryngology at the Queen's Medical Centre, Nottingham. Tissues were wrapped in saline-moist gauze and immediately delivered to the Pathology Department. Orientated mucosal samples had full-length strips of mucosa dissected off the lateral wall, and were then fixed in 10% formal saline and processed into paraffin wax. The mucosa was orientated so as to obtain transverse sections of the epithelium and full-thickness submucosa. Four micron thick sections were produced for immunohistochemistry (IHC) and *in situ* hybridization (ISH). Monoclonal antibodies against IgE (Dako, Cambridge, UK; clone CIA-E-7.12), plasma cells-CD138 (Serotec, Oxford, UK; clone B-B94) and mast cell tryptase (Dako; clone AA1) were used with a streptavidin peroxidase detection technique, and diaminobenzidine chromogen. Immunostained sections were counterstained with haematoxylin.

IgA, IgD, IgG and IgM antibodies (Dako) were used to assess the heavy chain immunoglobulin production in plasma cells and the predominance determined using a subjective scoring system based on the proportion of plasma cells showing Ig expression. The IgE antibody showed no cross-reactivity with IgA, IgG, IgM, kappa or lambda light chain paraproteins.

Two techniques were used for eosinophil detection. Carbol chromotrope staining [20] and an anti-major basic protein immunohistochemical technique were used. A similar empirical dye and immunohistochemical method was used to differentiate between granulated and non-granulated mast cells. Mast cell tryptase antibody detects granulated and degranulated (activated) mast cells whereas the metachromatic dye, toluidine blue, stains heparin granules in the granulated mast cells [21]. The number of degranulated mast cells was calculated by subtraction of the toluidine blue-stained cell mean from the mast cell tryptase mean for each stratum.

In situ hybridization

The monoclonal anti-CD138 IHC used for plasma cell detection was considered to be unreliable for the epithelial morphometry because of cross-staining with epithelial cells. To overcome this problem, an ISH technique was used. A fluorescein-labelled probe against kappa light chain immunoglobulin mRNA was used according to the supplier's instructions (Vector, Cambridge, UK). The sensitivity of the technique allowed predominantly plasma cell detection as these cells have an increased gene copy number (above 100) of secretory light chain immunoglobulin compared to mature B cells which express 20–40 copies [22]. The ISH-stained cells showed good

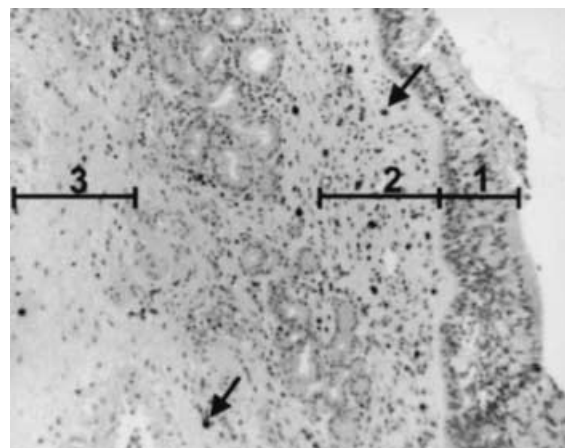


Fig. 1. Section of idiopathic rhinitic mucosa stained for IgE; positive cells are stained brown (arrowed). For morphometric analysis, the nasal sections were divided into strata comprising the full-thickness epithelium (stratum 1), superficial submucosa (stratum 2) and deep submucosa (stratum 3). ($\times 100$ magnification).

comparability with CD-138 IHC for plasma cells (Fig. 2). Comparison of the kappa ISH with immunohistochemistry for B cells (CD20) showed the two techniques detected different cell populations.

Morphometry

We performed morphometry on orientated whole bilateral sections (when available) of turbinate mucosa to determine the inflammatory cell populations. Sections were counted blind to their origin, and each section was divided into three strata comprising the epithelium, a superficial submucosa stratum immediately underlying the epithelium, and a third deeper submucosa stratum lying immediately adjacent but distal to the glandular region. The submucosal strata had a depth equivalent to the field diameter of a X16 objective lens (Fig. 1). Every other field was counted per section (average = 12 fields). Total cell counts were performed for the epithelium using a Kontron Videoplan Image Analysis System (Kontron Elektronik Ltd, Herts, UK). Cell counts were calculated per mm^2 .

Preliminary counts for mast cell tryptase-positive cells showed there was no significant difference between the left and right turbinates in all three strata ($P = 0.48$). Similarly, no significant difference was detected for cell counts along the turbinate length ($P = 0.75$). Cell counts were made for each stratum by counting every field and Mann–Whitney non-parametric analysis was used to compare the means of the middle third and each of the distal and proximal third values.

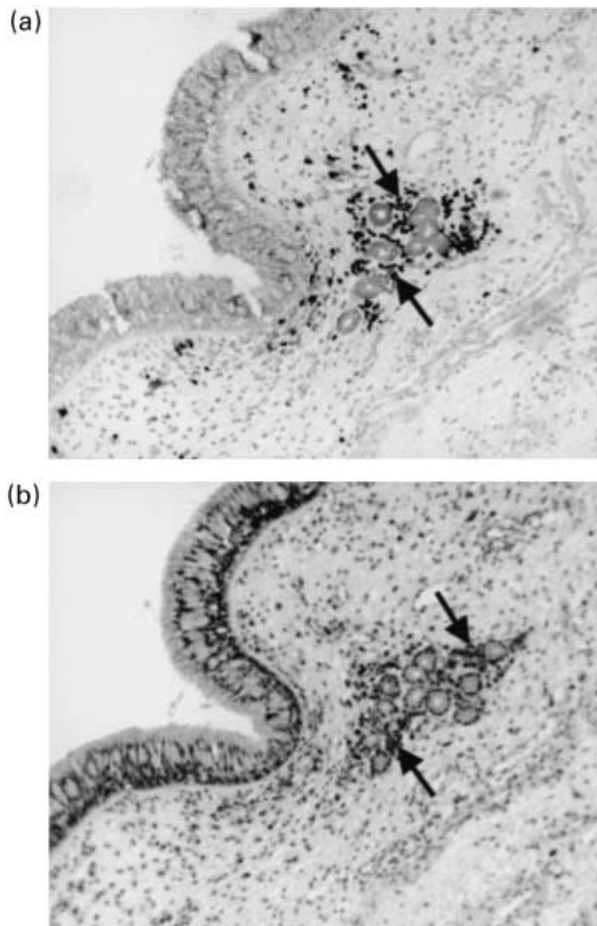


Fig. 2. Comparison of the CD138 immunostaining (a) with the kappa mRNA *in situ* hybridization (b) gave good correlation and allowed the latter to be used for detection of plasma cells within the epithelium. ($\times 100$ magnification).

Statistical analysis

Mann–Whitney non-parametric analysis was performed on the mean cell counts (per mm^2) for each stratum comparing the allergic (AR), idiopathic (IR) and normal mucosa groups using a Minitab (PA, USA) program.

Results

Mast cells

Total mast cell populations were similar for the AR and IR epithelium ($P > 0.05$) after staining with the anti-tryptase antibody. Both groups differed significantly from the normal control tissue in showing a 6-fold increase in their mean mast cell population ($P < 0.03$) (Table 2). Toluidine blue staining showed most of the epithelial mast cells were degranulated. All three groups were similar in showing fewer mast cells in

Table 2. Inflammatory cell numbers (Mean values shown/ mm^2)

		Epithelium	Superficial Mucosa	Deep Mucosa
Mast cell tryptase	AR	65	33	13
	IR	66	35	18
	N	10	37	16
Toluidine blue	AR	11	20	10
	IR	2	21	15
	N	0	19	13
Degranulated mast cells	AR	53	13	3
	IR	64	14	3
	N	10	18	3
IgE	AR	132	41	17
	IR	36	27	12
	N	1	11	4
Eosinophils	AR	0	42	2.3
	IR	0	35	0.5
	N	0	1	0
Plasma cells	AR	0	160	7
	IR	0	114	8
	N	5	119	8

the adjacent superficial and deep submucosa. Median cell counts for each stratum are shown in Fig. 3.

IgE positive cells

IgE⁺ cells were seen scattered throughout the stroma of the submucosa with numerous cells localized within the epithelium. Comparison of replicate sections stained with mast cell and plasma cell immunostaining suggested the majority of the IgE⁺ cells were mast cells. Both the AR and IR groups had a significantly increased population of epithelial and superficial mucosal IgE⁺ cells compared to normal mucosa ($P < 0.05$) (Table 2; Fig. 3). However, the allergic rhinitic mucosa had significantly increased IgE⁺ cell numbers compared to the idiopathic rhinitic mucosa ($P < 0.05$).

Eosinophils

Two staining techniques were used for determining eosinophil numbers and both showed eosinophils were almost exclusively localized in the superficial submucosa with only occasional eosinophils seen in the epithelium and deep submucosa. Following carbol chromotrope staining, the allergic and idiopathic rhinitic groups showed significantly increased eosinophil numbers compared to normal mucosa ($P < 0.05$). In contrast, when an immunohistochemical technique using an antibody for major basic

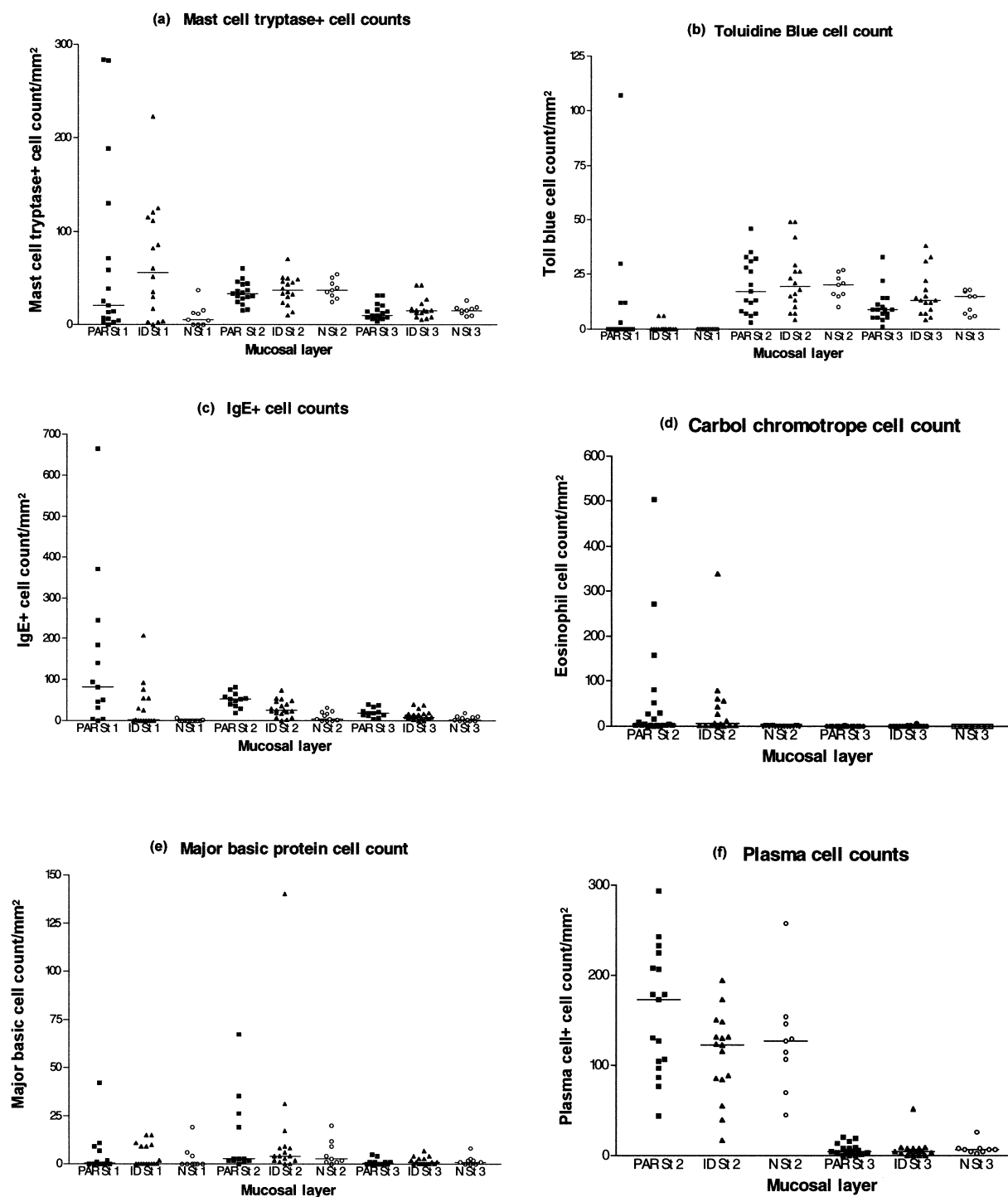


Fig. 3. Scattergrams showing the mean (a) mast cell, (b) Toluidine blue, (c) IgE⁺ cell, (d) carbol chromotrope (eosinophils), (e) major basic protein (eosinophils), and (f) plasma cell count/mm² for the perennial allergic (PAR), idiopathic (ID) and normal mucosa (N). The median value for each group is shown by a horizontal bar. The different mucosal regions counted were St1 = epithelium, St2 = superficial submucosa and St3 = deep submucosa.

protein was used, although increased eosinophils were demonstrated in the rhinitics compared to normal mucosa, the findings were not significant at the 5% level. Eosinophil counts are shown in Table 2 and Fig. 3.

Plasma cells

The anti-CD138 IHC showed similar numbers of plasma cells clustered around submucosal mucous glands in all three groups with a small but non-significant difference between allergic and idiopathic rhinitic mucosa ($P = 0.48$) (Table 2). Subsequent immunostaining on replicate sections revealed that the plasma cells mostly expressed IgA heavy chain immunoglobulin followed by IgG and IgD immunoglobulin. For the demonstration of epithelial plasma cells, three cases of each group were stained for kappa light chain mRNA using ISH. No staining was seen in the AR and IR groups but 5 cells were seen in the epithelium of one case of normal control tissue.

Discussion

In the current study we tested the null hypothesis that there would be no difference in the inflammatory infiltrate of nasal mucosa derived from idiopathic and perennial allergic patients. Our results failed to reject the null hypothesis for mast cell numbers with a power of 90%. We showed that activated (degranulated) mast cells, IgE⁺ cells and eosinophils were increased in perennial allergic and idiopathic rhinitis mucosa compared to normal non-rhinitic mucosa.

'Non-allergic' rhinitis has not been as comprehensively studied as allergic rhinitis for a number of reasons. Firstly, patients with non-allergic rhinitis are a heterogeneous group. Failure to consider this may explain the inconsistencies seen in previous studies when comparing non-allergic and allergic patient groups [23]. To improve on homogeneity, we applied strict selection criteria to obtain a group of patients with 'idiopathic rhinitis' as previously described [5]. For this reason, we rigorously excluded patients with autonomic rhinitis ('vasomotor rhinitis') and other forms of non-allergic nasal disease. To prevent any anomalies arising from quiescent seasonal allergies sampled out of season, we selected only perennial allergic rhinitics for study.

Direct comparison of previous rhinitic cellular infiltrates has been complicated by the range of sampling methods used including nasal brushings/scrapings [12,17], nasal washings [8,18,19] and mucosal biopsies [9,10,13,14]. By using whole full-thickness turbinectomy samples with an average length of 2.5 cm, we were able to reduce sampling error to a minimum. We found similarity in the local inflammatory infiltrate of left and right turbinates in

agreement with others [16] and along the length, though variation due to focal localization of cell populations was observed (data not shown). In contrast to previous studies relying on nasal brushing/washing analysis, our extensive analysis of strata within the mucosa allowed us to conclude that the epithelium and superficial lamina propria contained most of the cells involved in the Th2 type inflammatory response.

Mast cells and IgE⁺ cells

Increased mast cell numbers occur in allergic rhinitis [9,17]. Importantly, we showed that the allergic and idiopathic groups had a similar, increased population of epithelial mast cells compared to normal epithelium ($P > 0.05$). The majority of these cells were degranulated. The reason for the abundance of mast cells in the epithelium is not known but may involve differential cell migration from the submucosa to the epithelium [9]. An alternative explanation might involve selective diapedesis and maturation of juvenile mast cells at the local site of inflammation. Previously, Berger *et al.* [16] showed mast cells were predominantly localized in the submucosa but the apparent anomaly between the two studies can be explained by technical differences. In using toluidine blue staining alone, Berger was unable to detect degranulated mast cells which, in the present study, have been shown to be a feature of the epithelium.

We found increased epithelial IgE⁺ cell populations in AR and IR compared to normal epithelium ($P < 0.05$), with more IgE⁺ cells in the allergic group. Comparison with the mast cell results suggest that the perennial allergic group had a larger proportion of IgE⁺ cells which were not mast cells, compared to the idiopathic group. The most likely candidates are plasma cells as these were increased in the perennial allergic group and may produce IgE. Although we found that the majority of IgE⁺ cells were mast cells, the increased abundance of IgE⁺ cells over mast cells may be explained by binding to cells other than mast cells [24] including; macrophages, eosinophils, dendritic cells and a population of unidentified cells [25].

The clinical evidence for idiopathics being labelled non-allergic include negative serum RAST and skin prick tests, suggesting a lack of IgE involvement. Our study adds to the increasing evidence to challenge this definition of exclusion. Allergic-type responses have been noted in the absence of systemic atopy [23,26] and antigen-specific IgE [27]. Recent findings have shown that localized mucosal IgE switching is up-regulated in allergic rhinitic mucosa and selective B cell proliferation occurs [28,29]. Additionally, IgE production in B cells is increased in the presence of mast cells [30] as a result of IL-4 production [10] and IL-10 stimulation [31] confirming their

importance in allergic rhinitis. Taken with our observations, this suggests that a localized IgE-mediated Th2 inflammatory mechanism involving mast cells, may occur in idiopathic rhinitis without systemic IgE involvement.

There is evidence that nasal steroids decrease levels of IL-4 through a reduction of Th2 cells, predominantly eosinophils and T cells in allergic [32,33,34] and non-allergic rhinitis [35]. Mast cells are a source of IL-4 in perennial rhinitis [36] and, in showing these cells are significantly increased in AR and IR, we believe that the effect of steroids on these cells and their inflammatory mediators need further study.

Eosinophils

Eosinophils are believed to cause nasal epithelial damage and their numbers are known to increase during the pollen season [11,37]. Eosinophil numbers increase during late-phase allergic rhinitis responses [38] and since our rhinitis patient groups had chronic symptoms we predicted they would show a similar late-phase type response. In agreement with Berger *et al.* (1997)[16] we found not all the perennial allergic and idiopathic rhinitic cases had increased eosinophil numbers, suggesting eosinophilia is down-regulated in perennial-type rhinitis. We found eosinophils were predominantly localized to the superficial submucosa, with only occasional cells distributed within the epithelium and deep submucosa which is in agreement with previous studies [24]. On balance, both the allergic and the idiopathic rhinitic groups showed significantly increased eosinophil numbers compared to normal non-rhinitic mucosa using carbol chromotrope staining. Such a significant change was not detected using immunohistochemistry for eosinophil major basic protein. The fewer eosinophil numbers detected may be due to antigen masking induced by formalin fixation or a reduction in major basic protein-containing granules in post-late phase rhinitis. Previously, it has been reported that the size and number of eosinophil granules can change during activation [39], but eosinophils are not predominant in perennial allergic and idiopathic rhinitic turbinate tissue [16].

Plasma cells

The *in situ* hybridization technique for kappa light chain mRNA was used successfully to demonstrate plasma cells in the mucosal epithelium, overcoming the cross-staining encountered with the CD138 antibody. Although epithelial plasma cell localization was not a significant feature of any of the samples investigated, large populations of cells were seen in the glandular region of the submucosa. Recent attention has focused on the contribution of plasma cells to IgE production in allergic rhinitis but we did not find

obvious IgE localization to plasma cells in the limited number of samples we examined. We found that the predominant plasma cell heavy chain immunoglobulins were IgA, IgG and IgD.

Conclusion

There have been very few studies on the pathophysiology of idiopathic rhinitis. Our findings show that AR and IR have similar epithelial mast cell populations which differ from those found in the epithelium of normal mucosa. IgE⁺ cells were shown to be increased in both rhinitic groups compared to normal mucosa but more so in the allergic group. Both rhinitic groups had significantly increased numbers of eosinophils compared to non-allergic, normal mucosa. As mast cells and IgE⁺ cells have a key role in the Th2 IgE-mediated inflammatory response [10,40], we propose that idiopathic rhinitis may represent a form of allergic rhinitis not involving systemic atopy. In a separate rhinomanometry study, we provide further evidence to support the concept that idiopathics and allergics share a common disease mechanism [41]. In future studies we intend investigating cytokine gene expression to confirm IR shows a localized Th2-mediated pathophysiology and to investigate the possible mechanism of mast cell activation in IR.

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