

‘Entropy’: localized mucosal allergic disease in the absence of systemic responses for atopy

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Summary

Background The Th2 immune response in the nasal mucosa of subjects with allergic rhinitis is mediated by allergen-specific IgE. Moreover, these subjects show positive responses for markers of systemic atopy, including allergen-specific skin sensitivity and raised serum IgE titres. In contrast, idiopathic rhinitis (IR) subjects with similar histological nasal mucosal features differ in being defined as non-allergic because they have negative atopic responses.

Objective We hypothesized that it is possible to have an allergic Th2 disease pathway localized in the nasal mucosa of ‘non-allergic’ rhinitis subjects despite an absence of atopic responses.

Methods The presence of house dust mite and grass pollen-specific IgE antibodies was investigated in non-atopic ($n = 10$) and atopic ($n = 11$) subjects with persistent rhinitis and compared to normal ($n = 12$) control subjects. Biotin-labelled allergen was used to localize specific allergen-binding antibodies *in situ* in sections of nasal mucosa.

Results Grass pollen allergen binding was detected in the nasal mucosa of 3/10 non-atopic IR subjects but, in contrast, dust mite-specific antibodies were not detected. Specific antibodies were present in a total of 8/11 mucosal samples from the allergic group, but none was detected in normal control tissues.

Conclusion These findings support the concept of localized nasal allergy in ‘non-atopic’ rhinitis subjects. We propose the term ‘entropy’ to define this phenomenon and believe that this concept has a wider implication for localized allergic responses in other mucosal sites.

Keywords atopy, idiopathic, IgE, inflammation, mast cells, nasal mucosa, rhinitis

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Introduction

Atopic subjects show a predisposition for allergic disease. By definition, subjects with allergic rhinitis have an IgE-mediated [1] allergic response involving a Th2 inflammatory pathway [2, 3]. Induced by allergen, ‘immediate response’ rhinitis symptoms result from mast cell degranulation [4] caused by cross-linking of allergen-specific IgE on mast cells [5]. Allergic rhinitis subjects characteristically show affirmative responses to clinical tests for atopy, including allergen skin prick tests (SPTs), serum allergen-specific and increased total IgE levels. In spite of the reliance on diagnostic tests for atopy detection, the association between atopy and localized nasal symptoms is not understood.

The concept of localized mucosal allergic disease in the absence of systemic atopy is not new as previously, allergen-specific IgE has been detected in nasal secretions of non-atopic subjects with rhinitis [6]. Furthermore, when subjected

to allergen nasal provocation, a sub-group of non-allergic idiopathic subjects show nasal airway responses similar to those seen in allergic rhinitis [7]. It is known that the presence of atopy is not responsible for rhinitis, since passive induction of dermal atopic responses does not result in rhinitis symptoms [8]. Other studies also support a possible lack of association between markers of atopy and allergic disease. Epidemiological studies show that there is a lack of correlation between the prevalence of atopy and the level of serum IgE [9], and this might explain why 50% of allergic individuals have serum total IgE concentrations in the normal range [10]. Furthermore, longitudinal studies suggest that the incidence of allergen skin reactivity and (allergic) rhinitis symptoms may occur independent of each other [11].

In contrast to their atopic counterparts, subjects with persistent idiopathic rhinitis (IR) [12] are classified as non-allergic [13] because they have negative responses to clinical tests for atopy. Consequently, the disease pathophysiology of this group has been ascribed as unknown. In an attempt to investigate the disease pathway of this common group, we recently showed that a sub-population of idiopathic patients have a nasal mucosal inflammatory infiltrate similar to that seen in allergic rhinitis. The histology shows a prevalence of

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IgE⁺ cells, mast cells and eosinophils [14]. Subsequently, we hypothesized that some IR subjects have an allergic disease pathway localized to their nasal mucosa in the absence of clinical features for atopy. To test this, we used histochemical analyses [15] to detect intrinsic antigen-binding specific for house dust mite and grass pollen allergen in nasal mucosa from idiopathic and allergic rhinitis subjects, and normal controls.

Methods

Study subjects

Inferior turbinate specimens were surgically removed from consenting patients in the Division of Otorhinolaryngology at Queen's Medical Centre (QMC), Nottingham, UK. A total of 11 patients with persistent allergic rhinitis (PAR) (age range 17–52 years), 10 IR (age range 17–61 years) and 12 normal controls (age range 17–54 years) were rigorously selected according to strict criteria [14]. The IR tissues used in the present study represent a sub-selection of archival cases chosen because their nasal mucosa contain increased numbers of mast cells, IgE⁺ cells and eosinophils, verified by immunohistochemistry. Patients with a history of asthma, eczema, or autonomic rhinitis were excluded to improve on selection homogeneity. Clinical details of subjects involved in this study are shown in Table 1. None of the patients was clinically challenged with allergen prior to surgery. All

patients underwent surgery for nasal obstruction in and out of the main UK pollen season. Normal control nasal mucosa was obtained from patients operated on for mechanical obstruction secondary to a septal deviation. These patients were free of rhinitic disease pre- and post-operatively. Approval for this study was obtained from the QMC Trust's ethics committee.

Tests for atopy determination

Patients were tested for atopy using SPT and measurement of serum IgE levels. A panel of common allergens (Alk-Abelló, Hørsholm, Denmark) was used for SPT including house dust mite, cladosporium, grass and tree pollen mix, silver birch, cat and dog danders, and bird allergens. Serum-specific and total IgEs were measured in accordance with the supplier's *in vitro* diagnostic test system instructions (Pharmacia and Upjohn Diagnostics, Uppsala, Sweden). The normal adult range of IgE concentration is 0–114 kU/L.

Detection of allergen-specific IgE

Full-thickness nasal mucosa was snap-frozen in liquid nitrogen and stored at –80 °C until required. Six micron-thick frozen sections were cut and fixed in acetone at room temperature for 10 min. Replicate sections were stained to demonstrate grass pollen- or house dust mite-specific IgE antibodies using two separate detection techniques. Grass

Table 1. Clinical details for the (a) non-atopic idiopathic rhinitis and (b) perennial allergic rhinitis group

					Allergen-specific IgE ⁺ cells in mucosa (mm ²)			
					GP		HDM	
Patient	Age (gender)	Op date	SPT	Total serum IgE Conc (kU/L)	ABC	Tyr	ABC	Tyr
(a) Non-atopic idiopathic rhinitis group								
1	35 (M)	Jan	—	14.3	0	0	0	0
2	20 (M)	Feb	—	5.5	0	1–5	0	0
3	51 (M)	Mar	—	16.0	0	0	0	0
4	61 (M)	Mar	—	9.0	0	0	0	0
5	27 (F)	Jun	—	8.4	0	0	0	0
6	25 (F)	Jul	—	6.8	0	0	0	0
7	17 (M)	Aug	—	39.1	0	0	0	0
8	30 (M)	Sep	—	118.2	0	1–5	0	0
9	49 (M)	Nov	—	19.4	0	0	0	0
10	24 (F)	Nov	—	11.9	5–10	5–10	0	0
(b) Perennial allergic rhinitis group								
1	26 (M)	Feb	1,2	312.0	5–10	> 10	5–10	5–10
2	37 (M)	Feb	1,4	181.6	0	0	0	0
3	26 (M)	Feb	1,2	163.1	5–10	> 10	0	0
4	17 (M)	Mar	1,2,3	148.0	5–10	> 10	1–5	> 10
5	52 (F)	May	1	44.3	0	0	0	0
6	51 (M)	Aug	2,3a	82.4	0	0	0	0
7	16 (M)	Sep	1	31.9	0	0	1–5	1–5
8	38 (M)	Sep	2,3,4	230.6	> 10	> 10	0	0
9	18 (M)	Oct	1,2	183.4	5–10	5–10	5–10	5–10
10	26 (M)	Oct	1	267.3	> 10	5–10	1–5	1–5
11	32 (M)	Oct	1,2	220.6	5–10	5–10	5–10	> 10

Key 1: house dust mite, 2: grass pollen, 3: tree pollen, 4: cats, GP: grass pollen; HDM: house dust mite, ABC: avidin–biotin complex detection, Tyr: tyramide detection.

Note (a) This patient had an equivocal SPT for dust mite, but a clinical history suggestive of house dust mite sensitivity.

Main UK grass pollen season extends from May to August. House dust mite allergens are perennial, but peak in October–January.

pollen and house dust mite allergens were chosen for our assay because these have been reported to give the most significant prevalence of positive skin tests [16]. Firstly, a streptavidin–biotin complex (ABC)-alkaline phosphatase technique was used as reported previously [15]. Sections were incubated in a 1:10 dilution of the biotinylated dust mite (DP-1; DPC Ltd, Caernarvon, UK) or grass pollen (GP-1; DPC Ltd) allergen for 1 h (Table 2). The allergens were confirmed to be IgE specific by the suppliers (personal communication with DPC Ltd) and by competitive inhibition experiments that involved pre-incubating the allergens in different immunoglobulin class-specific antibodies prior to their use [17]. Subsequently, captured allergen was detected using goat anti-biotin antiserum (Sigma Ltd, Dorset, UK) diluted 1:50 in 0.05 M tris-buffered saline (TBS), pH 7.6 labelled with alkaline phosphatase, followed by amplification with ABC-alkaline phosphatase (Dako Ltd, Cambridge, UK) prepared according to the supplier's instructions. Visualization was accomplished using nitro blue tetrazolium dye (NBT) (Sigma Ltd) and sections were counterstained in Neutral Red dye (Nu-Stain, Nottingham, UK).

An alternative tyramide-based detection technique [18] was used to increase the allergen-capture assay sensitivity. After incubation in the biotinylated allergen for 1 h, sections were blocked for endogenous peroxidase activity in 0.3% hydrogen peroxide containing 0.1% sodium azide. Subsequently, the allergen was detected using a streptavidin peroxidase/biotin tyramide sandwich technique according to the supplier's instructions (GenPoint, Dako Ltd, Cambs, UK). Allergen localization was visualized in diaminobenzidine (DAB) dye and the sections were counterstained in Mayer's haematoxylin dye.

Double-staining techniques were used to immunophenotype the allergen-positive cells in five allergic and three IR cases. Briefly, grass pollen or house dust mite allergen-specific IgE was demonstrated using the ABC-alkaline phosphatase detection technique as previously detailed above. A peroxidase-labelled secondary antibody (Dako Ltd) was used to detect the primary antibody specific for either mast cell tryptase (Dako Ltd), B19 (Dako Ltd) for B cells, or CD138 for plasma cells (Serotec, Oxford, UK). Visualization was achieved using Nova Red (Vector Laboratories, Cambs, UK). Negative controls were performed for the ABC and tyramide histochemical techniques by omitting the biotinylated allergen.

Inappropriate biotinylated allergens comprising moth (Alastat i8) and chocolate (Alastat f105) were used to test for non-specific binding.

Stained sections from each subject were screened for the presence of allergen staining without prior knowledge of the subjects' clinical status. Allergen-specific IgE appeared as homogeneous blue or brown cytoplasmic staining with the NBT and DAB dyes, respectively. Morphometry was performed using interactive image analysis (AnalySIS program, SIS, Germany) to determine positively stained cells/mm².

Results

Detection of antibodies specific for house dust mite allergen

Using the ABC-alkaline phosphatase and NBT dye combination, cells showing captured house dust mite allergen appeared blue stained. Staining was localized to the cell membrane/cytoplasm. With the tyramide detection technique, cells with captured allergen were stained brown using DAB. Positively stained cells were mostly large mononuclear cells predominantly distributed throughout the upper layer of the nasal sub-mucosa with fewer cells present in the epithelium. Subsequent double-labelling techniques confirmed these to be mostly mast cells. Staining of the sub-mucosal glandular epithelium was seen not only in the test but also in the negative control sections and for this reason, it was difficult to conclude if the glandular epithelium is a site of allergen capture.

Dust mite-specific staining was seen in a total of 6/11 subjects with persistent allergic rhinitis with total agreement between the two histochemical techniques.

Neither the IR nor normal control subjects showed staining for mite allergen in their mucosa. In comparing the two histochemical detection techniques, the tyramide technique gave better contrast of staining than the alkaline phosphatase technique, making detection easier.

Detection of antibodies specific for grass pollen allergen

Cells capturing grass pollen allergen stained blue with the ABC technique, with the staining being localized to the cell membrane/cytoplasm. Similar to the staining seen for the mite allergen, positively stained cells were predominantly distributed throughout the upper layer of the nasal submucosa with a minority of cells seen in the epithelium.

Cells staining for grass pollen were seen in 7/11 subjects with allergic rhinitis, with the ABC and tyramide (Fig. 1a) histochemical techniques showing total agreement. In contrast, the position was different in subjects with IR. One subject (1/10) showed pollen-capture with the ABC technique, while the tyramide technique detected three subjects (3/10) with mucosal allergen-capture, giving a total of 3/10 subjects with allergen-specific antibodies (Figs 1b and c). Staining was absent from all 12 of the normal control patients and in the negative controls, who had the allergen omitted from the staining procedure.

Table 2. Allergens used to detect allergen-specific IgE

Panel	Allergens (biotinylated)
DP1M	<i>Dermatophagoides pteronyssinus</i>
	<i>Dermatophagoides farinae</i>
	<i>Dermatophagoides microceras</i>
	<i>Lepidoglyphus destructor</i>
	<i>Tyrophagus putrescentiae</i>
	<i>Glycophagus domesticus</i>
	<i>Euroglyphus maynei</i>
	<i>Blomia tropicalis</i>
	Orchard grass
	Meadow fescue
GP1M	Perennial rye grass
	Timothy grass
	June grass (Kentucky blue)

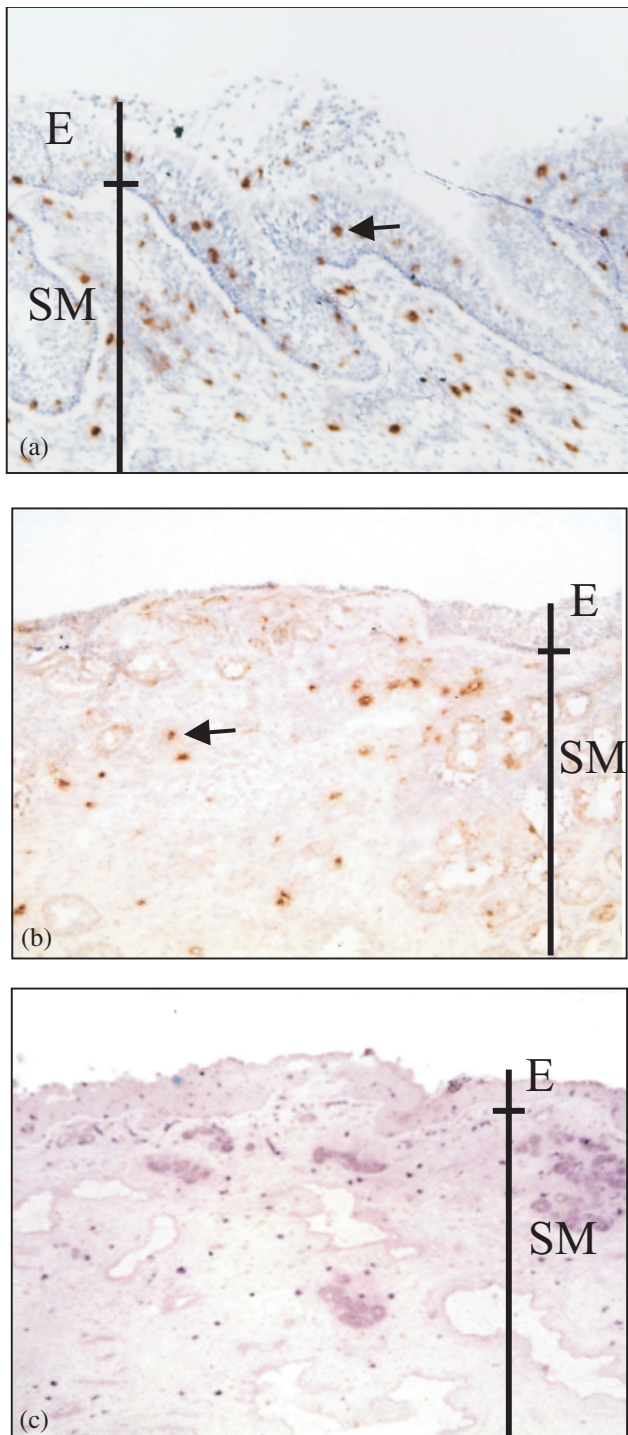


Fig. 1. (a) High-power detail from an allergic rhinitis subject with numerous grass pollen-specific mast cells traversing the nasal epithelium. Tyramide detection. E: epithelium, SM: sub-mucosa. (b) Full-thickness mucosa from a subject with non-atopic idiopathic rhinitis. Grass pollen is shown localized to cells throughout the mucosa using tyramide and (c) ABC-alkaline phosphatase detection techniques.

Co-localization of allergen-capture to B cells, plasma cells and mast cells

Double staining for allergen (pollen and mite)-capture with B cells, plasma cells, IgE or mast cells resulted in three staining

combinations. Where the allergen did not co-localize with the chosen cell type being demonstrated, separate blue and red staining was seen in the respective cell types. Red and blue (purple) staining was seen when co-localization of the allergen with the immunophenotyped cell type occurred. Some, but not all mast cells showed allergen-capture (Fig. 2a). Up to 20% and 10% of the mast cell population in allergic and idiopathic mucosa, respectively, showed co-localization with grass pollen allergen. Up to 3% of plasma cells co-localized with allergen. Negligible B cells showed allergen-capture (Fig. 2b).

Discussion

Previous studies suggest an independence between atopy and the ability to experience allergic-type symptoms [6, 11]. To test the hypothesis that localized allergy can occur in mucosal

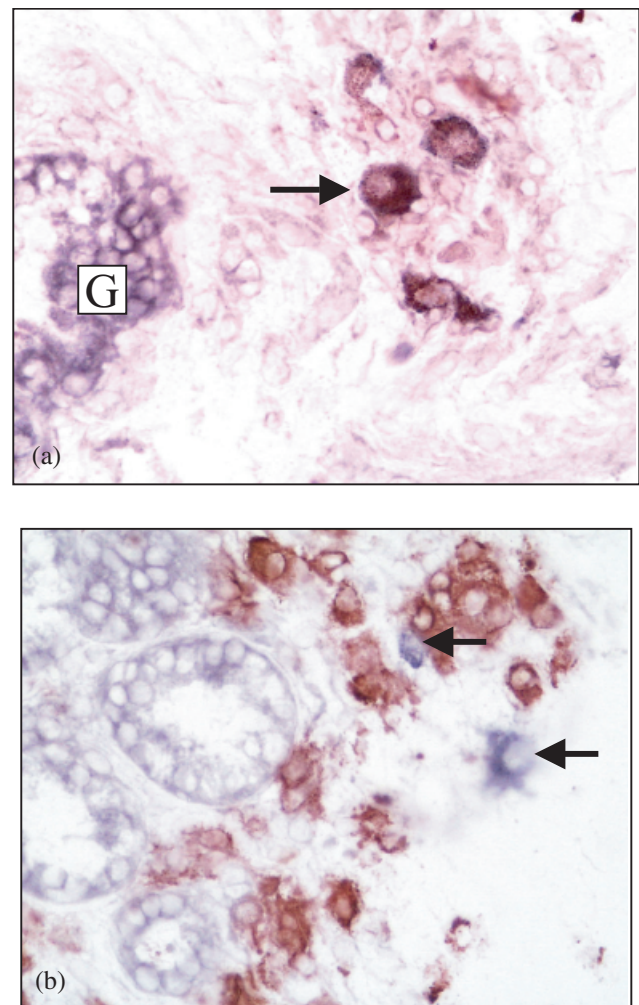


Fig. 2. (a) Double-labelling technique on nasal mucosa from an allergic subject shows purple/brown staining indicating co-localized grass pollen co-localized to a mast cell tryptase-positive cell. Non-specific binding of detection reagents to mucin was seen in glandular epithelium (G). (b) Very few plasma cells co-localized with grass pollen. Here, two allergen-positive cells (arrowed) are seen among a group of plasma cells (red) in the nasal mucosa of an atopic subject.

tissue independent of markers for atopy at distant tissue sites, we compared subjects with persistent allergic rhinitis to subjects with non-atopic IR. The latter group was selected because they had mucosal features compatible with an inflammatory pathology [19]. Importantly, our two study groups differed in being defined as atopic and non-atopic, respectively, on the basis of SPT and serum total/specific IgE for the assessment of atopy status. In line with expectations, staining for pollen or dust mite allergens was seen in the nasal mucosa of 7/11 allergic subjects. Confirming our hypothesis, we identified grass pollen capture in 3/10 idiopathic subjects. This evidence supports the concept that local mucosal inflammatory responses can occur independent of remote atopic responses. This is the first report of mucosal allergen-capture in non-atopic rhinitis subjects. Consequently, we propose the term '*entopy*' (Greek '*entopos*' – meaning local resident) to describe the phenomenon of a localized mucosal response independent of systemic atopic responses. We believe that this concept has a wider implication and may occur in allergic diseases of the skin, gastrointestinal tract, and eye.

Subjects with allergic rhinitis have a Th2 inflammatory disease pathway [3, 20, 21]. Activated T lymphocytes and their secreted cytokines induce immunoglobulin class switching in B cells [22] locally producing IgE [15, 23], while importantly maintaining antigen specificity [24]. Our findings confirm previous reports showing mucosal allergen binding [15] and indicate that allergen is co-localized to 10% and 20% of the mast cells from IR and allergic rhinitis subjects, respectively. Evidence of allergen uptake was not seen in the normal control group. The histochemical detection technique used for mucosal allergen-capture was shown to be both sensitive and specific with none of the negative controls giving false-positive results.

The relationship between nasal mucosal allergic disease and the phenomena causing systemic atopic responses is not understood. Subjects with non-atopic IR provide a good study group for investigating this association. In this study, we investigated a sub-selection of idiopathic subjects with a mucosal mast cell pathology similar to that found in their allergic counterparts. Previously, Blom et al. [25] reported two cases of non-allergic rhinitis with increased inflammatory cells, concluding that these were probably non-atopic 'allergic' subjects. Here, we showed that 30% of our non-atopic group had evidence of allergen-capture in their nasal mucosa, and we offer a number of possibilities to explain this finding. It is possible that localized nasal allergy precedes systemic atopy and if correct, non-atopic members would subsequently be expected to develop atopy. Most subjects in this cohort have had their disease for several years and we have no evidence to suggest that they subsequently develop atopy. Indeed, epidemiological studies suggest a reduction in allergen skin reactivity and rhinitis symptoms with time, independent of each other [11]. Moreover, IgE levels stabilize in middle-age rather than increasing with time [26].

A more plausible explanation for our findings is that we detected false-positive results due to cross-reactive carbohydrate determinants (CCD) [27]. CCDs are common antigenic structures shared by allergens from different taxonomical sources, including recognition between plants, foods and

arthropods. It is also common to find cross-reactivity ('pan-allergy') in patients for different arthropod species [28]. Previously, we reported that approximately 42% of IR subjects have mucosal evidence of an allergic pathway [14]. It is conceivable that these patients have allergic rhinitis induced by allergens other than those commonly used for SPT. Countering the CCD or pan-allergy possibility, we did not see allergen binding with inappropriate allergens for chocolate and moth (an arthropod) in our three grass pollen-positive non-atopic patients. Furthermore, if CCDs were responsible for the observed allergen binding in these cases, they would have been detected in the serum-specific IgE assays [27].

The absence of raised serum IgE in IR patients need not be a major concern, as IgE has been shown to be a relatively poor indicator of rhinitis [29]. Fifty per cent of subjects with allergic rhinitis have IgE in the normal range and between 11% and 22% of subjects with seasonal rhinitis show negative SPT responses to common allergens [10]. Furthermore, less than 50% of IgE-mediated allergic diseases are associated with atopy [30]. Here, we found anomalies between atopic markers and rhinitis in the allergic group. Three subjects with allergic rhinitis had total IgE concentrations in the normal range and yet one of these (Patient #7) had dust mite antibodies present in their nasal mucosa. The converse was also seen, including one atopic subject (Patient #10) with mucosal pollen antibodies but a negative skin test for the same allergen. In addition, another atopic subject (Patient #3) was mite-SPT positive, but specific antibodies for this allergen were not detected in their nasal mucosa using the allergen-capture technique.

Interestingly, our study also suggests that differential IgE longevity may occur, depending on the nature of the allergen causing the immune response. Here, we found clear evidence of grass pollen-specific IgE in patients operated on from October to February, outside of the recognized pollen season (May–August). The half-life of serum IgE is reported to be 2.5 days, but may persist for 8 weeks in allergic subjects [31]. Furthermore, the increased numbers of inflammatory cells seen in chronic rhinitis may be responsible for maintaining IgE production, along with pollen associated with house dust [32].

Excluding idiopathic subjects with entopy, 70% of our non-atopic rhinitis group had an unknown pathophysiology. In the entopic subgroup, we showed that allergen was co-localized to mast cells, compatible with the Th2 pathway and localized mucosal production of IgE. Our findings suggest that nasal allergen provocation might be a better test for determining allergy-associated rhinitis, especially as this technique has proved more sensitive than SPTs and serum allergy tests (RAST) [33].

Conclusion

Previously, we reported that 42% IR subjects have mucosal features suggestive of allergic disease, suggesting that these subjects might be incorrectly categorized as non-allergic. Here, in investigating selected non-atopic subjects with IR, we showed that up to 30% of this group have *entopy* – a nasal mucosal allergic disease in the absence of a positive SPT/serum IgE to the same allergen. This supports the concept that atopy and localized allergy can occur independently.

Moreover, we believe that this concept could apply to allergic responses seen in other mucosal sites, including the skin, gastrointestinal tract and eye.

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References

- 1 Kaur B. International study of asthma & allergies in childhood (ISAAC) phase II. South Thames. London, UK: NHS Executive, 1998.
- 2 Johansson S, Hourihane JOB, Bousquet J et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56:813–24.
- 3 Durham S, Ying S, Varney V et al. Cytokine messenger RNA expression for IL-3, IL-4, IL-5, and granulocyte/macrophage-colony-stimulating factor in the nasal mucosa after local allergen provocation: relationship to eosinophilia. *J Immunol* 1992; 148:2390–4.
- 4 Naclerio RM, Meier HL, Kageyabotka A et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 1983; 128:597–602.
- 5 Froese A. The presence of two kinds of receptors for IgE on rat mast cells. *J Immunol* 1980; 139:600.
- 6 Huggins K, Brostoff J. Local production of specific IgE antibodies in allergic rhinitis patients with negative skin tests. *Lancet* 1975; 2:148.
- 7 Carney AS, Powe DG, Huskisson RS, Jones NS. Atypical nasal challenges in patients with idiopathic rhinitis: more evidence for the existence of allergy in the absence of atopy? *Clin Exp Allergy* 2002; 32:1436–40.
- 8 Solley G, Gleich G, Jordan R et al. The late phase of the immediate wheal and flare skin reaction: its dependence upon IgE antibodies. *J Clin Invest* 1976; 58:408–20.
- 9 Burney P, Malmberg E, Chinn S et al. The distribution of total and specific IgE in the European Community Respiratory Health Survey. *J Allergy Clin Immunol* 1997; 99:314–22.
- 10 Sibbald B, Rink E. Epidemiology of seasonal and perennial rhinitis: clinical presentation and medical history. *Thorax* 1991; 46:895–901.
- 11 Simola M, Holopainen E, Malmberg H. Changes in skin and nasal sensitivity to allergens and the course of rhinitis; a long-term follow-up study. *Ann Allergy Asthma Immunol* 1999; 82:152–6.
- 12 Carney A, Jones N. Idiopathic rhinitis: idiopathic or not? *Clin Otol* 1996; 21:198–202.
- 13 Lund V. Report of the International Rhinitis Management Working Group. *Allergy* 1994; 91 (Suppl):1–34.
- 14 Powe D, Huskisson R, Carney A et al. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. *Clin Exp Allergy* 2001; 31:864–72.
- 15 Kleinjan A, Godthelp T, van Toornenbergen et al. Allergen binding to specific IgE in the nasal mucosa of allergic patients. *J Allergy Clin Immunol* 1997; 99:515–21.
- 16 Leech S, Lampe F, Johnston S et al. Which skin prick tests best identify atopy? Abstract presented at the British Society for Allergy and Clinical Immunology 2001
- 17 Kleinjan A, Vinke JG, Severijnen L et al. Local production and detection of (specific) IgE in nasal B-cells and plasma cells of allergic rhinitis patients. *Eur Respir J* 2000; 15:491–7.
- 18 Bobrow M, Harris T, Shaughnessy K, Litt G. Catalyzed reporter deposition, a novel method of signal amplification. *J Immunol Methods* 1989; 125:279–85.
- 19 Powe DG, Huskisson RS, Carney AS et al. Is idiopathic rhinitis a highly-localised form of allergic rhinitis, confined to the nasal mucosa? *J Allergy Clin Immunol* 2001; 107:784.
- 20 Varney V, Jacobson M, Sudderick R et al. Immunohistology of the nasal mucosa following allergen-induced rhinitis. *Am Rev Respir Dis* 1992; 146:170–6.
- 21 Calderon M, Lozewicz S, Prior A et al. Lymphocyte infiltration and thickness of the nasal mucus membrane in perennial and seasonal allergic rhinitis. *J Allergy Clin Immunol* 1994; 93: 635–43.
- 22 Gauchat J, Lebman D, Coffman R et al. Structure and expression of germline transcripts in human B cells induced by interleukin-4 to switch to IgE production. *J Exp Med* 1990; 172:463–73.
- 23 Durham S, Gould H, Hamid Q. Local IgE production in nasal allergy. *Int Arch Allergy Appl Immunol* 1997; 113:128–31.
- 24 Wang A, Wilson S, Hooper J et al. Evidence for control of synthesis of the variable regions of heavy chains of immunoglobulin chains G and M by the same gene. *Proc Natl Acad Sci* 1970; 66:337–42.
- 25 Blom H, Godthelp T, Fokkens W et al. Mast cells, eosinophils and IgE-positive cells in the nasal mucosa of patients with vasomotor rhinitis. An immunohistochemical study. *Eur Arch Otorhinolaryngol suppl* 1995; 1:S33–9.
- 26 Oryszczyn MP, Annesi I, Neukirch F et al. Longitudinal observations of serum IgE and skin prick test response. *Am J Respir Crit Care Med* 1995; 151:663–8.
- 27 Mari A, Lacovacci P, Afferni C et al. Specific IgE to cross-reactive carbohydrate determinants strongly affects the *in vitro* diagnosis of allergic diseases. *J Allergy Clin Immunol* 1999; 103:1005–11.
- 28 Panzani R, Ariano R. Arthropods and invertebrates allergy (with the exception of mites): the concept of panallergy. *Allergy* 2001; 56 (Suppl. 69):1–22.
- 29 Ng M, Warlow R, Chrishanthan N et al. Preliminary criteria for the definition of allergic rhinitis: a systemic evaluation of clinical parameters in a disease cohort (II). *Clin Exp Allergy* 2000; 30:1417–22.
- 30 Arshad SH, Tariq SM, Matthews S et al. Sensitization to common allergens and its association with allergic disorders at age 4 years: whole population birth cohort study. *Pediatrics* 2001; 108:U82–9.
- 31 Naclerio R, Adkinson N, Moylan B et al. Nasal provocation with allergen induces a secondary serum IgE antibody response. *J Allergy Clin Immunol* 1997; 100:505–10.
- 32 Fahlbusch B, Hornung D, Heinrich J et al. Predictors of group 5 grass-pollen allergens in settled house dust: comparison between pollination and nonpollination seasons. *Allergy* 2001; 56:1081–6.
- 33 Salzano FA. Specific nasal provocation test with powder allergen. *Allergy* 1997; 52:32–5.