Histamine and its release from nasal polyps: preliminary communication[1]

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Summary: Two hundred consecutive patients admitted for polypectomy had no evidence of an increased incidence of allergic disorders. Mast cell degranulation was found on transmission electron microscopy and this resulted in considerable quantities of histamine in polyp extracellular fluid (124–7300 ng/ml). RAST levels of allergen-specific IgE to house-dust mite and mixed-grass pollens were raised in 4 out of 28 cases in polyp fluid, and in only one matched serum. *In vitro* challenge of polyp tissue with allergen extract and anti-IgE suggested an IgE-mediated response in only 4 of 36 patients.

Introduction

Nasal polyposis is a common clinical condition which all ENT surgeons have considerable experience in treating, yet it remains a poorly understood disease. There are two current theories of pathogenesis: the infective and the allergic (Wilson 1976). Neither is without its limitations, but at present the allergic hypothesis is more widely accepted. Two published studies on the electron microscope findings in nasal polyposis have shown that mast cells are degranulated, but they did not comment on granule ultrastructure (Cauna *et al.* 1972, Busuttil *et al.* 1976). The aims of the present study were to show whether an IgE-mediated response might be responsible for the degranulation found in nasal polyp mast cells.

Methods

Clinical material

Two hundred consecutive patients who were admitted to the ENT Department, Addenbrooke's Hospital for polypectomy were studied. They were questioned on their nasal and past allergic history, which included hay fever, drug allergies, asthma and eczema together with skin test results to nine common allergens (house dust, house-dust mite, Cladosporium, Aspergillus, mixed grass pollens, feathers, cats, milk and eggs together with a negative control all produced negative Bencard). These data were placed on a questionnaire and evaluated statistically (Drake-Lee *et al.* 1984*c*).

Fine structure of mast cells in nasal polyps

Polyps from 6 patients were studied; they included patients both with and without asthma, hay fever and positive skin tests. Ultra-thin sections were made from tissue placed into Karnovsky's fixative and infiltrated with spurr resin. They were double stained with uranyl acetate and lead citrate (Gibbons & Grimstone 1963, Reynolds 1963, Drake-Lee *et al.* 1984*a*).

Free extracellular histamine

Polyp fluid was removed by coarsely mincing and microfuging polyp tissue (Donovan *et al.* 1970, Drake-Lee & McLaughlan 1982). The resulting fluid, together with matched sera from 52 patients, was stored at −20°C until analysed spectrofluorometrically for histamine (Evans *et al.* 1973). Thirteen patients had a history of asthma, 4 of whom also had an aspirin sensitivity; 5 had a history of hay fever; and 22 had positive skin tests to one or more allergens (Drake-Lee *et al.* 1984*b*).

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Radioallergosorbent test (RAST) levels
Twenty-eight of the patients who had antigen challenge had sufficient polyp fluid to perform RAST levels. The allergens tested were those used in skin tests for house-dust mite and mixed-grass pollens (Bencard). Following removal of preservative from skin-test allergen preparations by dialysis, allergens were coupled to cellulose filter paper (Ceska *et al.* 1972). Ten patients had asthma, 3 had hay fever and 9 had one or more positive skin tests.

Histamine release from polyp tissue compared with peripheral blood and skin tests
Extracts of house-dust mite and mixed-grass pollens were dialysed and diluted, 1 in 2, 1 in 5, and 1 in 10 tyrodes solution and, together with sheep antihuman IgE raised to myeloma PS, were used to challenge polyp tissue in a similar manner to that recently described (McLaughlan *et al.* 1983). Matched venous blood placed in a lithium heparin tube was also challenged with allergen extracts and sheep antihuman IgE (McLaughlan & Coombs 1983). Levels greater than 15% of total histamine available were considered as probably positive both for polyp tissue and peripheral blood (Norman *et al.* 1973, Radermecker 1980, McLaughlan & Coombs 1983). Fourteen patients had asthma, 4 hay fever and 13 had positive skin tests to one or more allergens (Drake-Lee & McLaughlan, in preparation).

Results

Clinical profile
Twenty-one patients (10.5%) had a history of hay fever and this was still present in two. There was a significant association between hay fever, penicillin allergy ($P<0.05$) and multiple positive skin tests ($P<0.001$). There appeared to be a genuinely atopic group, but they did not form a subgroup since they developed polyps at a similar age to non-atopic patients and did not have severe recurrence either.

Asthma was found in 29% of patients, being of childhood onset in 3.5% and late onset in the rest. Women were twice as likely to have asthma and polyps, but as expected there was a male predominance (3:1). Polypectomy had no effect on the asthma in the majority and in only one case did a patient complain that polypectomy had made the asthma worse; she developed asthma after her second polypectomy. The association between aspirin hypersensitivity (11 patients) and asthma ($P<0.001$) was confirmed.

Fine structure of mast cells in nasal polyps
A normal mast cell (Figure 1) is shown for comparison with those found in nasal polyps (Figure 2). It has large numbers of granules with an organized architecture and crystalline patterns (not seen at this magnification). Mitochondria are sparse, cytoplasmic cell projections may be seen and there is no microtubular system. In nasal polyps the basic cell structures were identified but the most striking feature was mast cell degranulation. There was a wide range of granule appearances from full ones to empty ones. The majority of granules contained material of differing densities and scroll patterns and crystalline structures were seen. A microtubular system was developed and large numbers of mitochondria were frequently found.

Free extracellular histamine
Histamine levels ranged from 124 to 7300 ng/ml, with a logarithmic and median value of 1103 and 1055 ng/ml respectively. The arithmetic mean was biased to the high end (1700). Values below 1000 were considered low and above 1000 high. There were no significant differences in histamine levels in patients with asthma, a history of hay fever, aspirin sensitivity and positive skin tests. The levels of corresponding paired sera were between 2 and 20 ng/ml with a mean of 10 ng/ml.

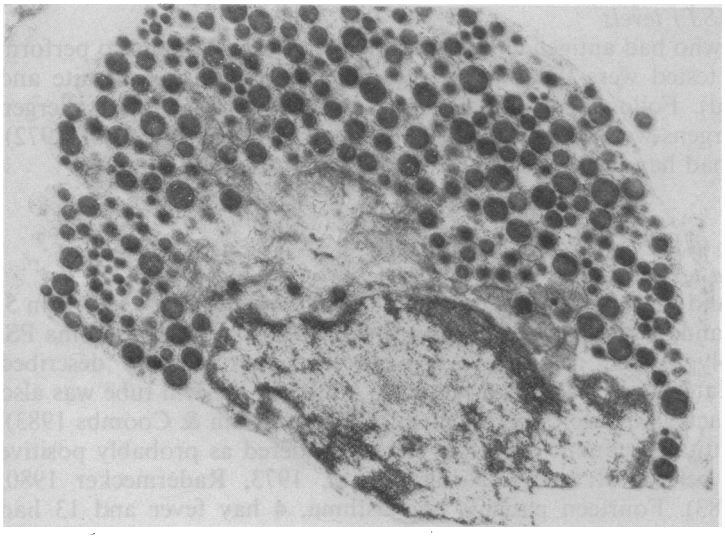

Figure 1. Normal nasal mast cell taken from the inferior turbinate (×6000). (Reproduced from Drake-Lee *et al.* 1984*a*, with kind permission)

RAST

Only 4 of 28 patients had a raised RAST in polyp fluid to either house-dust mite or mixed-grass pollens, which suggested little local production of allergen-specific IgE to the commonest inhaled allergens. Two patients had a raised RAST to house-dust mite and mixed-grass pollens, and another 2 to mixed-grass pollens alone. Two of the raised polyp RASTs to mixed-grass pollens had negative skin tests. Only one RAST in the matched sera was raised and that was to mixed-grass pollens.

Histamine release from polyp tissue compared with peripheral blood and skin tests

Polyp tissue released histamine less frequently than peripheral blood or skin test results would suggest. There was no correlation between polyp reactions and either skin test results or blood challenge (Table 1). A positive response was obtained with anti-IgE in 4 cases only.

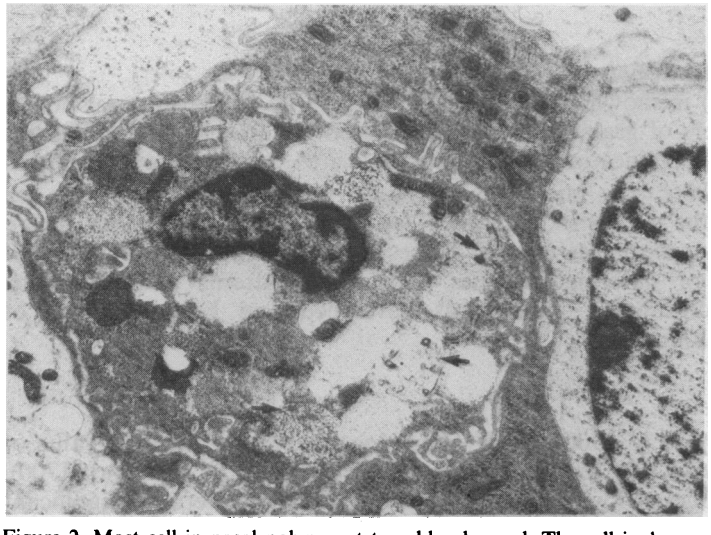

Figure 2. Mast cell in nasal polyp next to a blood vessel. The cell is degranulated to a variable degree and some granules contain scroll patterns (arrowed). Mitochondria are also present (×5000). (Reproduced from Drake-Lee *et al.* 1984*a*, with kind permission)

Table 1. Correlation between nasal polyp tissue and venous blood release of histamine with skin tests in 36 patients

Skin tests		Polyp		Blood	
		+ve	−ve	+ve	−ve
House-dust mite:	+ve	3	7	8	2
	−ve	5	21	2	24
Mixed-grass pollens:	+ve	1	5	4	2
	−ve	3	27	0	30

and in 3 of these there was antigen release as well. A total of 9 polyps released histamine on antigen challenge. Eight polyp patients released histamine with house-dust mite and 6 did so in the absence of a response to anti-IgE. Five patients released histamine with mixed-grass pollens (one had hay fever).

Peripheral blood and skin test results were of a similar order and were well correlated. Anti-IgE released histamine from peripheral blood in 14 cases and of these 7 were skin-test negative to allergens tested. Three patients who released histamine from blood with house-dust mite did so without a corresponding IgE response. One of the 4 who reacted with mixed-grass pollens also did not release histamine with anti-IgE.

Discussion

Polyps are considered allergic for three main reasons: the symptoms; the association with late onset asthma (Moloney & Collins 1977); and the histological findings which show a marked tissue eosinophilia in 90% of polyps (Friedmann & Osborne, 1982).

One of the main problems in considering the nature of any disease is agreement on the terminology. This is particularly true for 'allergic' disease. When the term 'allergy' was introduced by von Pirquet in 1906 it was used to describe the altered host reactivity to an antigen – in today's terms, most immune responses. It is now used both clinically and immunologically. At the cellular level 'allergy' is used to describe the tissue-damaging reactions which occur when two molecules of allergen-specific IgE situated on a mast cell at adjacent receptor sites react with a known allergen and cause the cell to degranulate. This can result in different symptoms at various sites. Unfortunately, similar symptoms of unknown aetiology are also labelled as allergies. It is best to restrict the use of the term 'allergy' to the IgE response, and this will be so here. The commonest allergens to cause nasal disease are mixed-grass pollens in seasonal rhinitis and house-dust mite in perennial rhinitis.

This confusion in usage of the word 'allergy' appears in the literature on nasal polyps. Some say that all patients with asthma are allergic (Samter & Lederer 1958) whereas others have suggested that allergy is uncommon (Caplin *et al.* 1971). Patients studied here do not appear to be any more allergic than expected by chance, and there is a genuinely atopic group (Drake-Lee *et al.* 1984*c*). This is supported by a study in which children with both polyps and cystic fibrosis were compared with children who had cystic fibrosis alone: this showed that eczema, asthma, hay fever, one or more positive skin tests and mean serum IgE levels were not significantly different between the two groups (Drake-Lee & Pitcher-Wilmott 1982). Since allergy does occur in patients with nasal polyps, allergic reactions are to be expected but should not commonly occur.

The mast cell degranulation (Drake-Lee *et al.* 1984*a*) did not seem to conform to *in vitro* changes found in human lung mast cells when degranulated by an IgE response (Caulfield *et al.* 1980). In nasal polyps the stages of degranulation were variable and the presence of crystalline structures and mitochondria suggested an ongoing process, perhaps not mediated through an IgE response.

The presence of considerable free histamine in polyp fluid in most cases confirmed that degranulation was still occurring. There was no difference or trend between polyp histamine

levels in patients with hay fever and positive skin tests, which suggested that an atopic subgroup did not exist.

The RAST to measure allergen-specific IgE was developed by Wide *et al.* in 1967. Our results confirm the general lack of raised RAST levels in polyp fluid and matched sera (John & Merrett 1979). In this study the same allergen preparations were used for skin testing, RAST and nasal challenge; this necessitated the preparation of RAST. The finding that there were only 4 patients in total who had raised allergen-specific IgE levels in polyp fluid to the commonest inhaled allergens demonstrated that, if the two commonest causes of allergic rhinitis contributed to nasal polyps, then they were an infrequent cause.

These findings were supported by the results from nasal challenge. Little work has been done on *in vitro* nasal challenge: histamine release has been studied in children with cystic fibrosis and nasal polyps (Kaliner *et al.* 1973). No prior sensitization was used in the 36 patients studied here. Nasal polyp tissue was less reactive than blood or skin tests would suggest. Challenge with an antihuman IgE suggested that there was insufficient cell-bound IgE present to initiate an IgE-mediated response in the majority of cases. Antigen-stimulated release of histamine in some cases in the absence of a response with anti-IgE may have indicated that other mechanisms were involved in histamine release.

The clinical features, RAST levels and the results of challenge to polyp tissue would suggest that allergic reactions may have occurred in nasal polyp patients but that these reactions did not occur frequently. They were unlikely to contribute to the evident mast cell degranulation seen on electron microscopy. Degranulation resulted in considerable quantities of free histamine present in polyp extracellular fluid.

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