

Metachromatic cells in nasal mucosa after allergen challenge

M. P. BORRES,¹ K. IRANDER² & B. BJÖRKSTÉN¹

¹Departments of Pediatrics and ²Oto-Rhino-Laryngology, Faculty of Health Sciences, Linköping University, Sweden

Metachromatic cells in the nasal mucosa were studied in relation to symptoms in 16 schoolchildren and 11 adults with hay fever who were challenged with pollen outside the pollen season, using either a gentle scraping-cytocentrifugation method for collection of mucosal specimens or biopsies. There was a temporary redistribution of metachromatic cells towards the mucosal surface appearing 5–24 h after challenge, with a correlation between the quantity of metachromatic cells and symptom scores. Thus, a single exposure to high doses of allergen may contribute to priming in susceptible individuals.

Key words: allergen challenge; metachromatic cells; nasal mucosa.

Accepted for publication 6 August 1989

CLINICAL ASPECTS

A gentle method to examine cells in the nasal mucosa in allergic children is described. Even a single exposure to allergen may induce redistribution of metachromatic cells to the nasal mucosa and thus prime for subsequent allergic reactions.

Metachromatic cells (MC) comprise basophils, connective tissue mast cells and mucosal mast cells in rodents (17). Heterogeneity of MC has also been found in humans and the cells are found in the mucous membranes including the upper respiratory tract (1, 5, 7, 12, 13, 16). There is a redistribution of MC from the sub-mucosa to the mucosa in response to natural exposure to pollen in hay fever patients (6, 18) and after nasal provocation tests (3, 10, 11). Different techniques have been used to collect cells from the nasal mucosa. Nasal lavage (2, 3, 14) and imprints (15) reflect conditions on the mucosal surface. With a curettage part of the epithelial layer is included, while biopsies allow studies of both the epithelial and the sub-epithelial layers (6, 12, 13). For studies in infants and small children a gentle method not needing an active collaboration by the patient is preferred, especially to obtain multiple specimens.

The aim of this study was to follow the cell response of MC and eosinophils kinetically in the upper airways after nasal challenge, using a gentle scraping-cytocentrifugation technique and biopsies.

MATERIAL AND METHODS

Sixteen school children, aged 10–18 years and 11 adults participated in the study. Material was obtained in the children by the scraping-cytocentrifugation technique while biopsies were performed in the adults. All the patients had suffered from allergic rhinoconjunctivitis during the birch or grass pollen seasons for at least 2 preceding years. All individuals were prick test positive to at least 10,000 BU/ml of birch or grass pollen extract. (Pharmalgen® or Phazet™, Pharmacia, Uppsala, Sweden). None of them had an infectious rhinitis at the time of

Table 1

Symptom scoring after nasal challenge with allergen			
Sneezes	Secretions (ml)	Air flow reduction (%)	Score
0	< 0.3	< 15	0
1-3	> 0.3	15-30	1
> 3	-	> 30	2

investigation, nor perennial allergic, non-allergic rhinitis or asthma.

Nasal challenge was performed with birch or grass pollen extracts (Pharmalgen® in March and April respectively, i.e. well before the pollen season. The extracts were sprayed into the nose in 0.2 ml amounts by means of a De Vilbiss spray No. 15, starting with dilution fluid. Pollen extract at a concentration of 100, 1,000 and 10,000 BU/ml was then administered at 10-min intervals. If clinical symptoms appeared after 1,000 BU/ml no more extracts were given except in five children who despite only minor symptoms received only 1,000 BU/ml in order to study the correlation between dose and cellular findings. The fluid was equally distributed to the right and left sides in the 16 children. As all the 11 adults were biopsied on the right side before the provocation, only the left side was accessible. Consequently, their challenged nostril received a higher cumulative dose than the non-biopsied children.

The number of sneezes, the amount of nasal secretions and nasal obstruction were recorded and scored according to Table 1. Nasal obstruction was evaluated by means of a Wright peak flow meter (Clement Clarke International Ltd., London, UK) adapted to an anesthetic mask. The means of three peak values from forced nasal inspiration were calculated. As shown in Table 1 a maximum symptom score of 5 could be obtained in each patient.

Mucosa scrapings for cell analysis were obtained with a wooden peg, gently rubbed against the inferior concha. The material was immediately placed in PBS (phosphate buffered saline, pH 7.3) and subsequently cytocentrifuged (Cytospin 2, Shandon, UK) for 5 min at

Table 2

Scoring of maximal increase cells/slide of metachromatic cells and eosinophils after nasal allergen provocation		
Metachromatic	Eosinophils	Score
0	0	0
< 4	< 20	1
5-19	21-199	2
> 20	> 200	3

500 rpm, after which the sediment was applied to glass slides and air-dried. The slides were either fixated in 95 % ethanol for 15 min and stained with Wright's stain for 2 min to visualize the eosinophils, or fixated in methanol and simultaneously stained with toluidine blue for 5 min to visualize MC.

Mucosal biopsies were taken from the inferior concha 1 cm behind the frontal edge in local anesthesia, using a conchotome with a diameter of 2 mm. The biopsy material was fixated in an iso-osmotic solution of formaldehyde and acetic acid (IFAA) and stained with toluidine blue according to Enerbäck et al. (6) to visualize MC.

To study the appearance of metachromatic and eosinophilic cells in the nasal mucosa, scrapings were taken bilaterally prior to the challenge, and after 30 min, and 1, 3, 5, 24 and 48 h in the 16 schoolchildren. Biopsies were taken from the right inferior concha before the challenge and from the left challenged side 5 or 24 h later in six and five adults respectively. The analysis of the samples was performed in light microscopy with all specimens coded. When studying the kinetics of MC and eosinophils, the maximal increases in cell numbers in each patient were scored according to Table 2.

Statistical method used was Spearman's rank correlation coefficient for the correlation between symptom scores and cellular findings.

All patients were fully informed about the procedure and the purpose of the study and gave their informed consent to participate. The study was approved by the Regional Ethics Committee for Human Research at the University Hospital of Linköping.

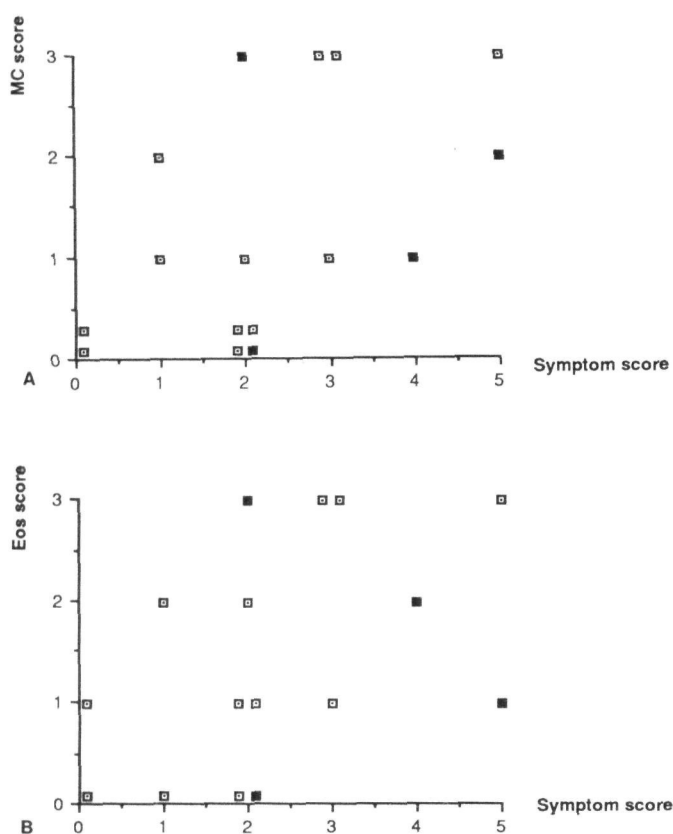


Fig. 1. Relation between increase in metachromatic (A) and eosinophilic (B) cells and symptom scores after nasal challenge. Symptom and cell scores are defined in Tables 1 and 2. The individuals were challenged with up to 1,000 BU/ml (open symbols) or up to 10,000 BU/ml (closed symbols).

RESULTS

Cellular findings in relation to symptoms

There was no correlation between cellular findings in specimens obtained before challenge and symptoms after the provocation tests, as the variation in symptom scores was similar in the 10 children without MC and the six with MC present in the mucosa before the challenge. A similar lack of correlation was noted in the biopsy specimens from the adult patients. Similarly, there was no correlation between eosinophils present before challenge and clinical symptoms after challenge.

Twelve children received extract at a concentration of 1,000 BU/ml and four children received 10,000 BU/ml. Symptom scores were evenly distributed in these two groups. As shown in Fig. 1, there was no correlation be-

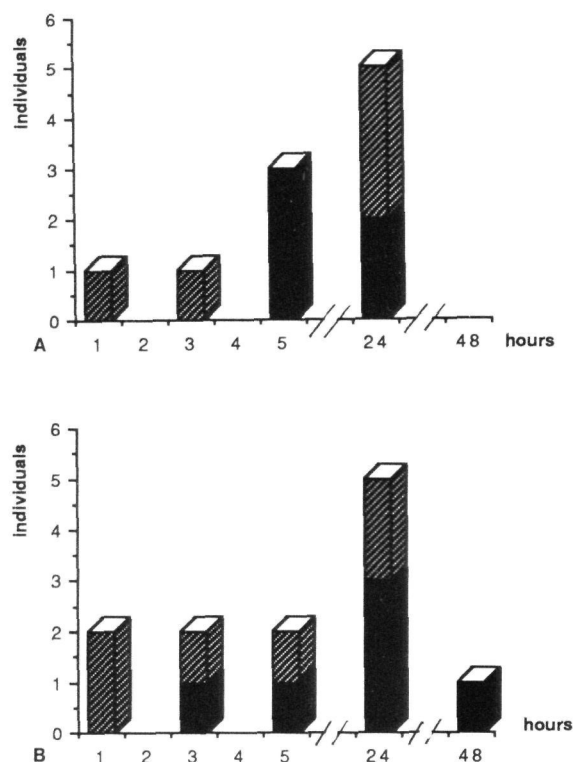


Fig. 2. Appearance of peak number of metachromatic cells (A) and eosinophils (B) in 12 individuals after nasal challenge with allergen. Closed symbols indicate individuals with a symptom score of at least two. In two subjects only eosinophilic and no metachromatic cells were seen.

tween dose amount and cell score, i.e. maximal outflow of MC or eosinophils after challenge. There was, however, a weak but statistically significant correlation between the symptom score and cell score ($r = 0.64$, $P < 0.01$ for MC and $r = 0.55$, $P < 0.05$ for eosinophils), (Fig. 1 A, B). This means that children who developed pronounced symptoms after challenge also tended to have a large increase in MC and eosinophil numbers compared to individuals with fewer clinical symptoms.

Kinetics of MC

All but two individuals had less than three MC in their nasal scrapings before challenge. Ten children reacted upon challenge with a MC increase. The highest counts appeared after 5 to 24 h in eight of them, including the five children with a symptom score of two or more (Fig. 2). In two subjects, the maximum counts were recorded after 1 to 3 h.

In the biopsy material most of the MC were found in the submucosa with occasional MC in

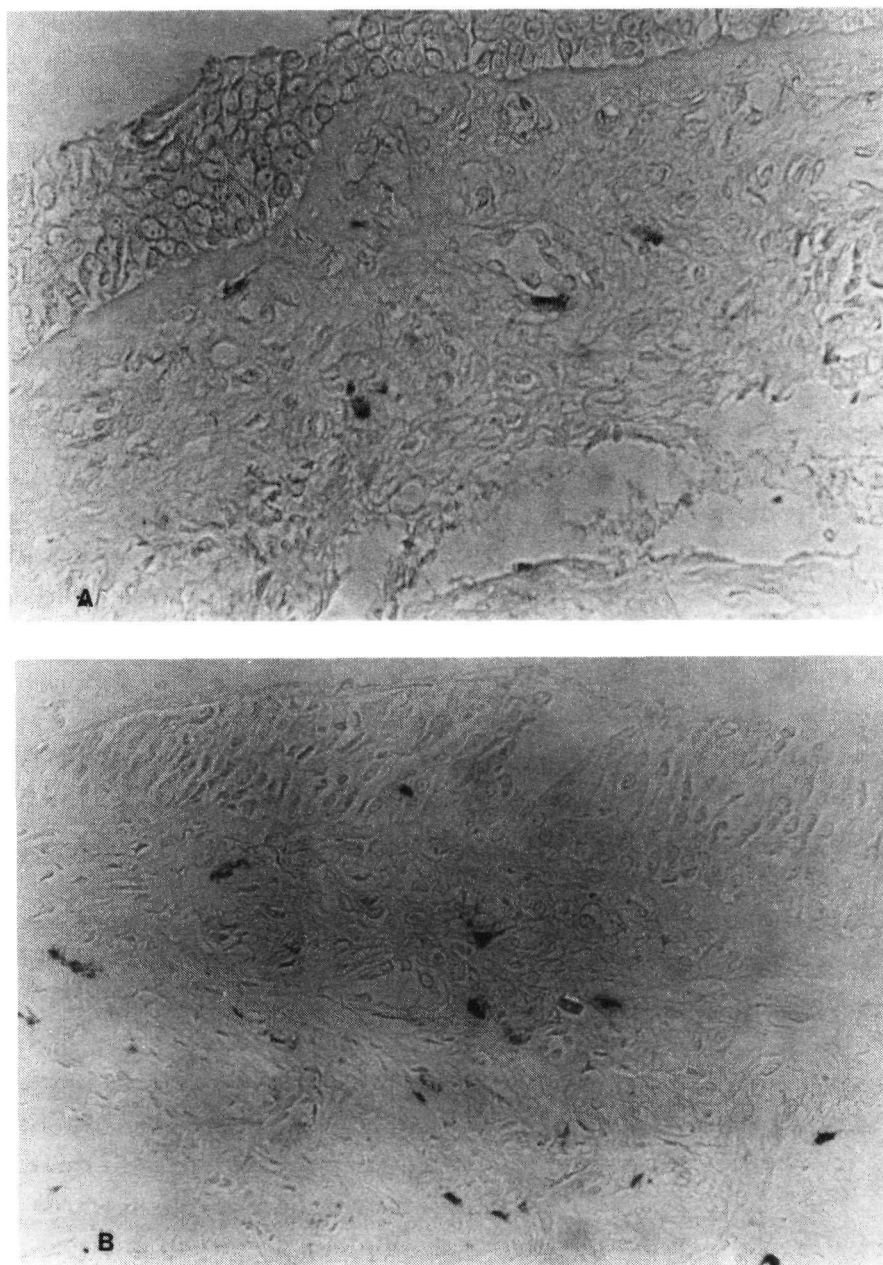


Fig. 3. Nasal biopsies stained with toluidine blue showing metachromatic cells before provocation (A) and 4 h after (B). Magnification $\times 25$.

the epithelium only in four patients. After challenge there was almost a doubling of MC numbers in the submucosa in three of the patients who were subject to a second biopsy after 5 h. No change in MC amounts was found in biopsies taken 24 h after challenge. These findings would indicate a transient redistribution of MC.

Kinetics of eosinophils

All but one of the children had less than 30 eosinophils per slide before challenge. Twelve of them reacted with increase in eosinophil

numbers upon challenge, with maximum cell counts 1 to 48 h after a positive reaction. The appearance over time varied more for eosinophils than for MC. (Fig. 2 B).

Three of the six children with no MC increase had no eosinophil increase and the other three had a slight increase (cell score one).

Relation MC and eosinophils

There was an agreement between the two cell scores after challenge in nine patients. In the remaining six, there was a difference in cell

scores of one. This points to a relationship between the two cell types ($r = 0.84$ $P < 0.01$), in the cellular response after nasal allergen challenge.

DISCUSSION

In this study a gentle technique for obtaining cells from the nasal mucosa, i.e. a scraping-cyto centrifugation method, was used in children, while biopsies were performed in the adults. Several techniques have been suggested for collecting material from the nasal mucosa (6, 12, 13, 14, 15). Since the number of MC is low in comparison with epithelial cells, neutrophils and eosinophils (13), the material available for MC analysis must not be too sparse. All sampling techniques however, are associated with some discomfort for the patient. The fact that cooperation is needed for nasal lavage and biopsies makes these methods less suitable for repeated sampling in young children. The imprint method (15) is based on egg albumin as an adhesive. This should be avoided since it represents a major allergen in children (9). Thus, many aspects must be considered when choosing an appropriate method, where the desire for abundant material must be balanced against the age and cooperativeness of the patient.

By the scraping-cyto centrifugation method material is collected from the epithelial surface on a more limited area of the nasal mucosa than with nasal lavage. The obtained material may therefore be less than after lavage, which may result in some underestimation of the number of MC. As a consequence, even low numbers of MC in the scraping samples are of interest.

An advantage of the scraping-cyto centrifugation method was a localized and clean material without disturbing mucus, which facilitated the microscopic examination of the obtained specimens. None of the children complained of discomfort when subjected to this method. It was difficult to estimate the true amount of secretions, as some was blown away or swallowed by the young patients. This made it necessary to use a scoring system which was less precise than that suggested previously in a study in adults (19).

Studies in adults with hay fever have shown that there is a redistribution of MC during the pollen season (6, 18) and after allergen challenge in a symptom-free interval (3). This study shows a similar redistribution of MC and eosinophils towards the surface of the mucosal membrane after a single allergen exposure. The redistribution is transient with increased numbers of MC on the surface of the mucosa for up to 48 h after challenge.

No correlation was found between pre-challenge MC findings and symptom scores, but there was a correlation between the number of cells appearing after challenge and the symptoms. No control patients were included, but it has been shown that allergen challenge has no influence on eosinophils in non-allergic persons (2).

In conclusion, this study shows that a scraping-cyto centrifugation technique is useful when a gentle method allowing repeated analysis is needed, e.g. for studies in young children. The absence of MC in a specimen, however, does not exclude their presence in the mucosa. For use in infants the scraping-cyto centrifugation technique would be the preferred method. A single allergen exposure increased the numbers of MC and eosinophils in challenged areas for up to 48 h. For the MC there appeared to be a redistribution of cells towards the surface. Even a single exposure to allergen thus results in an outflow of MC on the surface of the mucous membrane, which might contribute to the priming effect (4, 8).

ACKNOWLEDGEMENTS

The technical assistance of Kerstin Hagersten, Lisbeth Hjälle and Lena Lindell is gratefully acknowledged. This study was supported by the Swedish Medical Research Council (Project 7510), The County of Östergötland and Pharmacia Diagnostics, Uppsala.

REFERENCES

1. Barrett, K. E. & Metcalfe, D. D.: Heterogeneity of mast cells in the tissue of the respiratory tract and other organ systems. *Am. Rev. Respir. Dis.* 135, 1190-1195, 1987.
2. Bascom, R., Pipkorn, U., Lichtenstein, L. M. & Naclerio, R. M.: The influx of inflammatory cells into nasal

- washings during the late response antigen challenge. *Am. Rev. Respir. Dis.* 138, 406-412, 1988.
3. Bascom, R., Waschs, M., Naclerio, R. M., Pipkorn, U., Galli, S. J. & Lichtenstein, L. M.: Basophil influx occurs after nasal antigen challenge: Effects of topical corticosteroid pretreatment. *J. Allergy Clin. Immunol.* 81, 580-589, 1988.
 4. Connell, J. T.: Quantitative intranasal pollen challenge. III. The priming effect in allergic rhinitis. *J. Allergy* 43, 33-38, 1969.
 5. Enerbäck, L., Okuda, M. & Dolovich, J.: Basophil/metachromatic mediator cell heterogeneity in nasal allergy. In Mygind, N. & Pipkorn, U. (eds.): Allergic and vasomotor rhinitis, pathophysiological aspects, pp. 121-135. Munksgaard, Copenhagen, 1987.
 6. Enerbäck, L., Pipkorn, U. & Graneus, G.: Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int. Arch. Allergy Appl. Immunol.* 80, 44-51, 1986.
 7. Gomez, E., Corrado, O. J. & Davies, R. J.: Histochemical and functional characteristics of the human nasal mast cell. *Int. Arch. Allergy Appl. Immunol.* 83, 52-56, 1987.
 8. Grönborg, H., Borum, P. & Mygind, N.: Histamine and methacholine do not increase nasal reactivity. *Clin. Allergy* 16, 597-602, 1986.
 9. Hattevig, G., Kjellman, B., Johansson, S. G. O. & Björkstén, B.: Clinical symptoms and IgE responses to common food proteins in atopic and healthy children. *Clin. Allergy* 14, 551-559, 1984.
 10. Kawabori, S., Okuda, M., Unno, T. & Nakamura, A.: Dynamics of mast cell degranulation in human allergic nasal epithelium after provocation with allergen. *Clin. Allergy* 15, 509-515, 1985.
 11. Okuda, M. & Otsuka, H.: Basophilic cells in allergic nasal secretions. *Arch. Otorhinolaryngol.* 214, 283-289, 1977.
 12. Okuda, M., Sakaguchi, Y., Suzuki, F., Ohtsuka, H. & Kawabori, S.: Ultrastructural heterogeneity of the basophilic cells in the allergic nasal mucosa. *Ann. Allergy* 54, 152-157, 1985.
 13. Otsuka, H., Denburg, J., Dolovich, J., et al.: Heterogeneity of metachromatic cells in human nose: Significance of mucosal mast cells. *J. Allergy Clin. Immunol.* 76, 695-702, 1985.
 14. Pipkorn, U., Bisgaard, H. & Wihl, J.-Å.: Nasal provocation testing and lavage technique. In Mygind, N. & Pipkorn, U. (eds.): Allergic and vasomotor rhinitis, pathophysiological aspects. pp. 149-164. Munksgaard, Copenhagen, 1987.
 15. Pipkorn, U. & Enerbäck, L.: A method for the preparation of imprints from the nasal mucosa. *J. Immunol. Method* 73, 133-137, 1984.
 16. Schulman, E. S., MacGlashan, D. W., Schleimer, R. P., et al.: Purified human basophils and mastcells: current concepts of mediator release. *Eur. J. Respir. Dis.* 64, (Suppl. 128), 53-61, 1983.
 17. Shanahan, F., Denburg, J. A., Bienenstock, J. & Befus, D.: Mast cell heterogeneity. *Can. J. Physiol Pharmacol.* 62, 734-737, 1984.
 18. Viegas, M., Gomez, E., Brooks, J. & Davies, R. J.: Changes in nasal mast cell numbers in and out of the pollen season. *Int. Arch. Allergy Appl. Immunol.* 82, 275-276, 1987.
 19. Wihl, J.-Å.: Methodological aspects of nasal allergen challenges based on a three-year pollen immunotherapy study. *Allergy* 41, 357-364, 1986.

Address:

Bengt Björkstén

Department of Pediatrics

Faculty of Health Sciences

University of Linköping

S-581 85 Linköping

Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.