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IgE-positive mast cells on the human nasal mucosal surface in response to allergen exposure

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SUMMARY

IgE-bearing mediator cells are suggested to be the effector cells in type I allergic rhinitis. These cells can be demonstrated by their granular constituents or by the surface-bound JgE antibodies. We developed immunohistochemica! techniques in order to stain the cell-bound JgE using polyclonal or monoclonal anti-human IgE antibodies. These techniques can be applied to nasal biopsies as shown previously or to cytospin specimen harvested by a brush method. They deliver excellent staining results with well-preserved morphological details.

Brush samples were taken from 24 grass pollen allergic subjects before season, after a nasal allergen provocation and two weeks after the onset of season. There was a statistically significant increase in toluidine blue positive and JgE-positive cells 24 hours after nasal provocation (app. 12-fold, $p < 0.05$) and more pronounced within the season (app. 58-fold, $p < 0.001$) compared to preseasonal values. These cells appeared to be mast cells rather than blood basophilsjudged by morphological criteria. There was a striking correlation between the number of toluidine blue cells and that of JgE-positive cells ($r = 0.98$). The number of eosinophils also increased due to the seasonal allergen exposure ($p < 0.001$), but less pronouncedly compared to the mast cells. These data re-emphasize the migration of JgE-bearing mast cells and eosinophils into the epithelial lining of the nasal mucosa due to allergen interaction and point to a possible role of mast cells as a carrier for JgE-molecules.

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INTRODUCTION

Accumulating evidence point to the mast cell as a central triggering cell of allergic inflammation not only in nasal challenge setting but also during natural allergen exposure (Plaut and Lichtenstein, 1978; Naclerio et al., 1983; Norman et al., 1985; Enerback et al., 1986; Gomez et al., 1986; Pipkorn et al., 1988; Bachert et al., 1989). Evidence has been gathered from observations on the human nose such as changes in numbers and activation of mast cells or release of mast cell mediators into the nasal secretion. lgE bound to the cell surface of mediator cells is also of central importance and initiates the degranulation of the cells by an interaction between inhaled allergen and allergen-specific IgE. So far there have mainly been studies on IgE circulating in the serum in nasal secretions (Mygind et al., 1975; Merrett et al., 1976; Platts-Mills, 1979), but not associated to cells. We have recently,,shown that in allergic rhinitis patients cells can be stained for their membrane-bound lgE in nasal biopsies using monoclonal (mab) or polyclonal (pab) anti-IgE antibodies (Bachert and Ganzer, 1987; Ganzer and Bachen, 1988). lgE-positive cells in the human nasal mucosa resemble mast cells rather than blood basophils of plasma cells, their number significantly increases due to natural allergen exposure and the cells migrate into the epithelium due to allergen interaction (Ganzer and Bachert, 1988; Bachert et al., 1989).

As nasal biopsy represents a traumatic procedure for the patient which can not be performed repeatedly as it causes bleeding and may lead to inflammatory reactions and scaring of the mucosa, we intended to transfer our immunohistochemical techniques to a simple non-traumatic method for continuous monitoring of cellular events. From our previous results we expected the epithelial lining and the mucosa! surface to be the compartments of interest in order to investigate_the migration oflgE-positive cells. We therefore extended the techniques for the visualization of cell-bound IgE to a cytological sampling method the brush method described by Pipkorn et al. (1988).

The aim of this study was to establish a technique for the immunohistochemicul demonstrition of lgE on human mucosa! surface cells by poly- or monoclonal antibodies and to correlate these data with the staining results of metachromatic cells. For this purpose we investigated epithelial cells in 24 grass pollen allergic subjects before season, after nasal allergen provocation and within the season using the nasal brush method.

PATIENTS AND METHODS

Patients

Specimen were obtained from 24 grass pollen allergic patients (14 female, 10 male, average age 26.5 years) with a hay fever history of at least three years. The first specimen were harvested in a symptom free state during the pollen free winter month, the second 24 hours after a nasal allergen provocation test

(lyophilized and standardized grass pollen allergen mixture, 10,000 BE/ml, Allergopharma, Reinbek, F.R.G.) on the challenged nostril and the third two weeks after the onset of hay fever symptoms within the grass pollen season. The study was approved by the Ethics Committee at the University of Mannheim.

Sampling technique

The rnucosal surface cells were harvested with the brush method detailed elsewhere (Pipkorn et al., 1988) and placed in a plastic tube containing 1.2 ml 0.9 % sodium salt solution. In each case 0.4 ml of the cell containing solution were then centrifuged at 1500 rpm for 10 min in a cytocentrifuge and air-dried. One of the specimen was stained with toluidine blue at pH 0.5 for 30 min, another with hematoxylin-eosin and the third for IgE.

lmmunohistochemical techniques

Using specimen of allergic patients not included in this study the appropriate antibody concentration were investigated by checkerboard titrations. Procedures omitting the primary antibody or replacing it by unspecific antibodies served as controls. In eight patients the IgE-staining by pab was compared to that by mab using specimen from the same brush sampling.

For the polyclonal anti-human IgE-staining a preincubation with methanol-H_2O_2 for 20 min and normal swine serum 1: 10 for 10 min at room temperature was useful. Interrupted by washing procedures in PBS buffer, the specimen were incubated with anti-human IgE antibodies (Dakopatts, Hamburg) diluted 1: 600 for one hour at room temperature, with the goat anti-rabbit antibody diluted 1 : 50 for 30 min and the PAP-rabbit complex diluted 1:200 (Dakopatts) for 30 min. The specimen were stained using 3.3-diarninobenzidine for 10 min. The cells were counterstained with hemalaun.

The monoclonal anti-human IgE antibody (Bioscience, Switzerland) was diluted 1: 100 and incubated for one hour without preincubations. After a washing Process the rabbit anti-mouse antibody (Dakopatts) diluted 1 : 50 was used and followed by the mouse APAAP complex diluted 1: 50 (Dakopatts) for another 30 min. The specimen were stained using a Fast Red-Naphthol **AS-MX** solution With levamisole for 30 min at 37 °C and counterstained with hemalaun.

Cell counts were performed by light-microscopic evaluation at a magnification of 200x to 500x by one investigator on coded sections. For photographs a Kodak 50 ASA professional film was used. For statistical analyses the Wilcoxon-signed rank test was used on a $p < 0.05$ and $p < 0.001$ level.

RESULTS

The cells harvested by the brush method were well preserved with excellent structural details. Both staining techniques, the polyclonal anti-IgE antibody

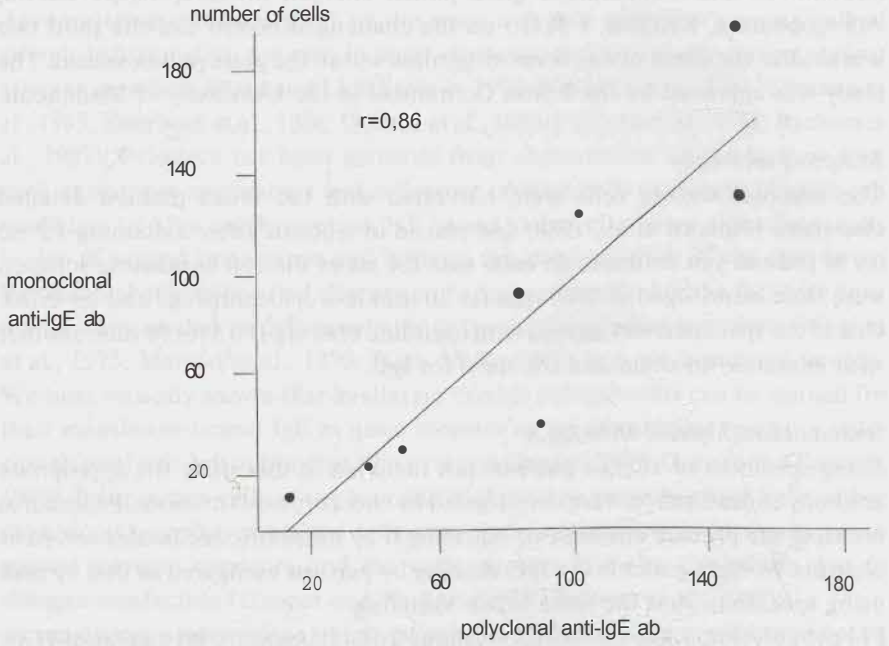

Figure 1. The number of cells stained with the polyclonal antibody correlates well to that stained with monoclonal anti-IgE antibodies (r = 0.86).

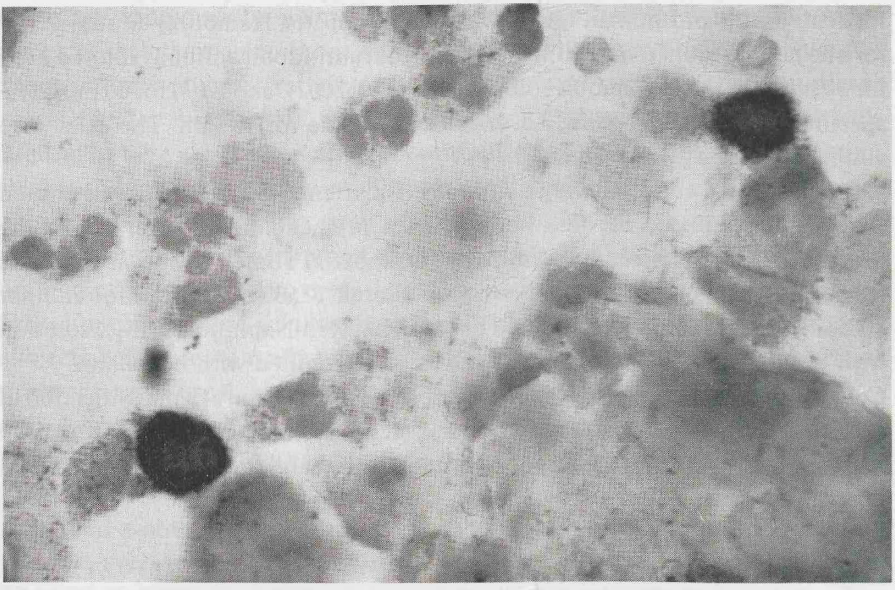

Figure 2. Cells stained with the monoclonal anti-IgE antibody are clearly distinguishable (magnification x425).

with the PAP-complex and the monoclonal antibody with the APAAP-complex, lead to clearly visible and specific staining results on the cell surface. The comparative staining of specimen ($n = 8$) showed an excellent correlation between pab positive and mab positive cells ($r = 0.86$) (Figure 1). As the monoclonal anti-IgE antibody gave more brilliant and distinguishable staining results (Figure 2), this technique was chosen for further investigations.

There was a statistically significant increase in the number of IgE-positive cells ($p < 0.05$) from before season to after nasal provocation and more clearly from before season (with or without provocation) to within season ($p < 0.001$) (Figure 3). Whereas in the symptom free interval in 18 out of 24 subjects (75 %) no IgE-positive cells could be found in the specimen, only one object was negative within the season with some subjects showing up to 300 IgE-positive cells at that time. Overall there was a 12-fold increase of IgE-positive cells after the nasal provocation and a 58-fold increase due to the natural allergen exposure.

The number of toluidine blue stained cells (pH 0.5) behaved synchronously to that of the IgE-positive cells. Again there was a significant increase in cells after nasal provocation ($p < 0.05$) and under natural allergen exposure ($p < 0.001$) (Figure 3).

In all cases there was a striking correlation between toluidine blue cells and IgE-stained cells with a coefficient of $r = 0.98$ in the specimen obtained within the season (Figure 4).

More than 90 % of the toluidine blue cells and the IgE-positive cells were mast cells rather than basophils judged by morphological criteria. The cells had a round or ovoid shaped nucleus and clearly more granules than blood basophils. The IgE-staining was localized on the surface membrane of the cells, covering the cells completely in some cases.

The number of eosinophils increased as a tendency after the nasal provocation and significantly within the season ($p < 0.001$) compared to the preseasonal values.

DISCUSSION

With the nasal brush method and the immunohistochemical technique for the visualization of surface IgE developed for nasal cytology we were able to demonstrate the migration of IgE-positive mediator cells into the nasal epithelium due to artificial and natural allergen exposure.

In recent studies we investigated IgE-positive cells in human allergic mucosa using biopsy specimen (Bachert and Ganzer, 1987; Ganzer and Bachert, 1988; Bachert and Baum, 1989; Bachert et al., 1989). Nasal biopsy is a traumatic procedure with inconvenience for the patient which may lead to unspecific inflammatory reaction and scaring of the mucosa and may thus influence following biopsies. We intended to change from nasal biopsy to a method which allows

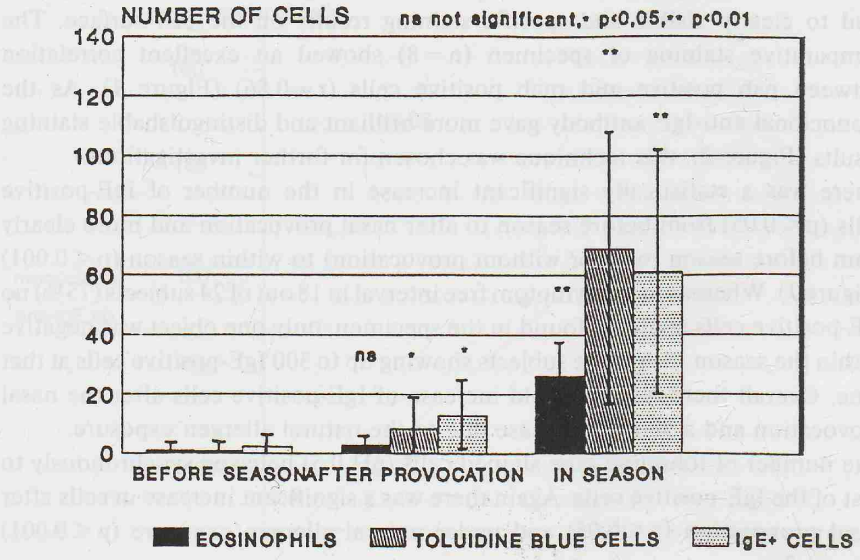

Figure 3. The nasal allergen provocation outside the season led to an approximately 12-fold increase of IgE-positive and toluidine blue cells in the brush samples. The natural allergen exposition caused a striking increase of mast cells, IgE-positive cells and eosinophils compared to preseasonal values.

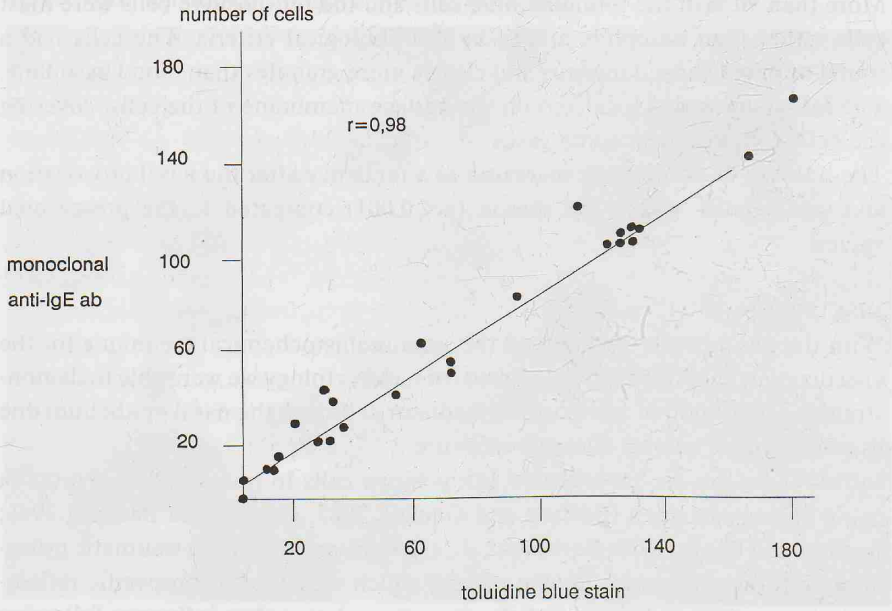

Figure 4. There was an excellent correlation between the IgE-positive and the toluidine blue stained cells (r=0.98).

repeated atraumatic samplings of cells from a compartment of the mucosa where we could expect dynamic cellular reactions involved in the mucosal allergic response. Referring to previous studies our interest focussed on the epithelial lining and the mucosal surface, where we could demonstrate IgE-positive mediator cells penetrating the basal membrane during natural allergen exposure, but not outside the allergen season (Bachert et al., 1989). Events taking place on the surface of the nasal mucosa can be studied using nasal smears, imprints, lavage procedures or the brush method (Hansel, 1953; Okuda and Otsuka, 1977; Hastie et al., 1979; Pipkorn and Enerbäck, 1984; Enerbäck et al., 1986; Pipkorn et al., 1988; Bachert and Baum, 1989). Both the lavage and imprint method recover cells from the epithelial surface and the mucous blanket, whereas the brush method harvests cells within the epithelial lining, as indicated by the finding of a high proportion of epithelial cells. As the whole epithelial lining above the basal membrane was the area of interest, we chose the brush method for cell sampling. In order to obtain reproducible sampling results, we standardized the brush method concerning the localization, the depth of introduction and the number of turns of the brush.

So far, most of our knowledge concerning localization, migration or numbers of mast cells has derived from studies using staining techniques for the visualization of the granular constituents (Okuda and Otsuka, 1977; Enerbäck et al., 1986; Viegas et al., 1987; Pipkorn et al., 1988). As the staining of mast cell subtypes is strongly dependent on the tissue fixation used, problems arise concerning the detection of mast cells especially of the mucosal subtype (Enerbäck, 1986; Pipkorn et al., 1988). Furthermore, mast cells which are totally degranulated due to allergen exposure, may not be detected with commonly used staining methods (Claman et al., 1987; Pipkorn et al., 1988). Thus a large proportion of mast cells may escape detection. This phenomenon can be overcome by using another property of mast cells such as the surface IgE bound by high affinity receptors. By checkerboard titrations and blocking of unspecific binding sites we were able to establish both a poly- and monoclonal anti-human IgE-staining technique on cells harvested from the epithelial layer of the human nasal mucosa. Both techniques gave reproducable and comparable results in terms of the numbers of positive cells ($r = 0.86$). We preferred the monoclonal anti-IgE staining using the alcaline phosphatase as labeled enzyme because of the excellently distinguishable and precise staining results with the cell nucleus still visible in most of the cells. Corresponding to the fact that in cytological specimen the cell surface of the whole cell can be reached by the anti-IgE antibody, the optimal concentration of the primary antibody was lowered to 1 : 10 or 1 : 20 compared to biopsy specimen. Using air-dried specimen without any additional fixation procedure we avoided fixation problems for the toluidine blue staining. The good correlation between toluidine blue and IgE-positive cells confirms the advantage of

this technique. This correlation furthermore points to the fact that the mediator cells were neither degranulated due to allergen exposure nor due to the sampling technique.

Judged by morphological criteria, the IgE-positive cells as well as the toluidine blue positive cells resembled mast cells rather than blood basophils. In contrast to nasal biopsy specimen we were not able to routinely perform double stainings using conventional mast cell stains and the anti-IgE technique in cytological specimen (Ganzer and Bachert, 1988). Therefore the identity of IgE-positive cells can only be concluded first from the fact that their number correlated extremely well with the number of toluidine blue cells and second from their morphology. We have to bear in mind, however, that eosinophils or macrophages may bind IgE with the help of a low affinity receptor (Capron et al., 1981; Melewicz et al., 1982). IgE-positive eosinophils can be excluded in our study because of their typical biloped nucleus which has not been seen in IgE-positive cells. The number of macrophages did not behave synchronously with that of IgE-positive cells. Futhermore, no cells could be stained for the low affinity IgE-receptors (unpublished data), so that there is no evidence for IgE-bearing macrophages in our specimen.

The number of IgE-positive cells in the mucosal epithelial layer of grass pollen allergic subjects clearly increased due to a solitary artificial allergen exposure outside the season and even more pronouncedly due to the natural allergen interaction during the pollen season. The increase was approximately 12-fold after the nasal provocation and approximately 58-fold two weeks after the onset of symptoms due to the natural pollen exposure showing a strong correlation to the number of toluidine blue cells. These results are consistent with previous studies demonstrating the "re-distribution" of toluidine blue cells into the epithelium (Pipkorn and Enerbäck, 1984; Enerbäck et al., 1986).

The study presents further evidence for the migration of mast cells through the epithelium into the nasal secretion. In contrast, no evidence for the trans-epithelial migration of basophils was obtained.

The increase of the number of the eosinophils within the epithelial lining was in accordance with previous reports (Bascom et al., 1986; Pipkorn et al., 1988) and points to a possible effector function of that cell as well. In this study the eosinophil number proved to be a good control parameter showing a less pronounced increase during the season compared to that of IgE-positive mast cells.

With the IgE-bearing mast cell being the target cell of the allergen/IgE-interaction, this cell migration strongly suggests an immunological explanation for at least part of the increasing reactivity of the mucosa to repeated allergen exposure (Konno et al., 1981; Borum et al., 1983) or the so-called priming effect (Connell, 1969). There is a strong increase in the number of target cells on the nasal surface

easily achievable by the allergen due to formerly allergen exposure. The migration of IgE-positive cells may as well serve as a model for the secretory mechanism of IgE-antibodies through the nasal epithelium (Bachert, in press). Furthermore it can be suggested that the chemotaxis of mast cells due to the allergic inflammation may be associated with the transport of (allergen-specific) IgE-antibodies from outside into the target organ, leading to an accumulation of IgE. This accumulation was formerly interpreted as a local IgE-production (Okuda, 1975; Merrett et al., 1976).

In conclusion the immunohistochemical demonstration of IgE on the surface of effector cells in the IgE-mediated allergic response appears to provide further valid information on changes of IgE-associated cell populations due to artificial as well as natural allergen interactions. Combined with commonly used mediator cell stains it may help to focus on cells immunologically involved in the allergic reaction by their surface IgE within the target organ. Our findings re-emphasize the role of the IgE-bearing mast cell in the pathogenesis of allergic airway disease and point to a further involvement of that cell as a carrier for IgE-molecules. The techniques of visualization of IgE in tissues and cell sampling may furthermore help to investigate the localization of IgE-synthesis and transport.

REFERENCES

1. Bachert C, Ganzer U. Staining of IgE-associated cells in the atopic nasal mucosa by the immunoperoxydase technique. Lar Rhinol Otol 1987; 66: 573–576.
2. Bachert C, Becker W, Ganzer U. The role of nasal secretions in allergic disease of the nose. Archs Otorhinolar 1989; 246: 173–182.
3. Bachert C, Baum M. NARES and nasal mastocytosis as differential diagnosis of allergic rhinitis. Allergologie 1989; 7: 282–286.
4. Bachert C, Marquardt U, Korte M. IgE-containing mast cells play a central role in nasal allergic disease. Am J Rhinol, in press.
5. Bascom R, Pipkorn U, Gleich G, Lichtenstein LM, Naclerio RM. Effect of systemic steroids on eosinophils (EOS) and major basic protein (MBP) during nasal antigen challenge (Abstract). J Allergy Clin Immunol 1986; 77: 246.
6. Borum P, Grönborg H, Brofelt S, Mygind N. Nasal reactivity in rhinitis. Eur J Resp Dis 1983; 64, Suppl 128: 65–71.
7. Capron M, Capron A, Dessaint JP, Torpier G, Johanson CGO, Prin L. Fc receptors for IgE on human and rat eosinophils. J Immunol 1981; 126: 2087–2092.
8. Claman HN, Giorno R, Choi KL. Degranulated ("phantom") mast cells in GVHD revealed by double immunofluorescence (Abstract). J Allergy Clin Immunol 1987; 77: 180.
9. Connell JT. Quantitative intranasal pollen challenges. III. The priming effect in allergic rhinitis. J Allergy 1969; 34: 33–39.
10. Enerbäck L. Mast cell heterogeneity: The evolution of the concept of a specific mast cell. In: Befus AD, Bienenstock J, Denburg J (Eds). Mast Cell Differentiation and Heterogeneity. Raven Press, 1986: 1–25.
11. Enerbäck L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. Int Archs Allergy Appl Immunol 1986; 80: 44–51.

12. Enerbäck L, Pipkorn U, Olofsson A. Intraepithelial migration of nasal mucosal mast cell in hay fever: ultrastructural observations. Int Archs Allergy Appl Immunol 1986; 81: 289.
13. Ganzer U, Bachert C. Localization of IgE-synthesis in immediate-type allergy of the upper respiratory tract. ORL 1988; 257–264.
14. Gomez E, Corrado OJ, Baldwin DL, Swanston AR, Davies RJ. Direct in vivo evidence for mast cell degranulation during allergen-induced reactions in man. J Allergy Clin Immunol 1986; 78: 637–645.
15. Hansel JK. The cytology of the secretions in allergy. In: Hansel FK (Ed). Clinical Allergy. St. Louis: CV Mosby, 1953.
16. Hastie R, Heroy JH, Levy DA. Basophil leucocytes and mast cells in human nasal secretions and scrapings studied by light microscopy. Lab Invest 1979; 49: 541–554.
17. Konno A, Togawa K, Nishihira S. Seasonal variation of sensitivity of nasal mucosa in pollinosis. Archs Otorhinolar 1981; 232: 253–261.
18. Melewicz FM, Kline LE, Cohen AB, Spiegelberg HL. Characterization of Fc-receptors for IgE on human alveolar macrophages. Clin Exp Immunol 1982; 49: 364–369.
19. Merrett TG, Houri M, Mayer ALR. Measurement of specific IgE-antibodies in nasal secretion evidence for local production. Clin Allergy 1976; 6: 69–73.
20. Mygind N, Weeke B, Ullmann S. Quantitative determination of immunoglobulins in nasal secretion. Int Arch Allergy 1975; 49: 99–107.
21. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Meyers DA, Norman PS, Lichtenstein LM. Mediator release after nasal airway challenge with allergen. Am Rev Resp Dis 1983; 128: 597–602.
22. Norman PS, Naclerio RM, Creticos PS, Togias A, Lichtenstein LM. Mediator release after allergic and physical nasal challenges. Int Archs Allergy Appl Immunol 1985; 77: 57–63.
23. Okuda R. IgE and IgE-antibodies to mite in nasal fluid. ORL 1975; 37: 344–355.
24. Okuda M, Otsuka H. Basophilic cells in allergic nasal secretions. Archs Otorhinolar 1977; 214: 283.
25. Pipkorn U, Enerbäck L. A method for the preparation of imprints from the nasal mucosa. J Immunol Methods 1984; 73: 133.
26. Pipkorn U, Karlsson G, Enerbäck L. Secretory activity of nasal mucosal mast cells and histamine release in hay fever. Int Archs Allergy Appl Immunol 1988; 87: 349–360.
27. Pipkorn U, Karlsson G, Enerbäck L. Phenotypic expression of proteoglycan in mast cells of the human nasal mucosa. Histochem J 1988; 20: 519–525.
28. Pipkorn U, Karlsson G, Enerbäck L. The cellular response of the human allergic mucosa to natural allergen exposure. J Allergy Clin Immunol 1988; 82: 1046–1054.
29. Pipkorn U, Karlsson G, Enerbäck L. A brush method to harvest cells from the nasal mucosa for microscopic and biochemical analysis. J Immunol Methods 1988; 112: 37–42.
30. Platts-Mills TAE. Local production of IgG, IgA and IgE-antibodies in grass pollen hay fever. J Immunol 1979; 122: 2218–2225.
31. Plaut M, Lichtenstein LM. Cellular and chemical basis of the allergic inflammatory response. Component parts and control mechanism. In: Middleton E, Ellis E, Reed CE (Eds). Allergy: Principles and Practice. St. Louis: CV Mosby, 1978: 115–138.
32. Viegas M, Gomez E, Brooks J. Davies RJ. Changes in nasal mast cell numbers in and out of the pollen season. Int Archs Allergy Appl Immunol 1987; 82: 275–276.

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