

Original Article

Differences in mast cell-bound IgE in the nasal epithelial layer and lamina propria

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ABSTRACT

Mast cells in the allergic nasal mucosa are reported to have higher concentrations of high affinity type I Fcε receptors (FcεRI) than mast cells in the non-allergic mucosa. IgE binds to FcεRI on mast cells. However, it is not clear whether different zones within the nasal mucosa harbor different quantities of IgE-bound mast cells. We stained IgE and mast cells from the nasal mucosa of patients with allergic and non-allergic rhinitis using a double-labeling technique with anti-IgE and antitryptase antibodies and compared the intensities of IgE staining of mast cells. In the allergic nasal mucosa, mast cells displayed more IgE staining in the lamina propria than in the epithelial layer. Mast cells displayed more IgE-staining in the allergic nasal mucosa than in the non-allergic mucosa. We speculate that the quantity of IgE that binds to mast cells depends on the quantity of IgE in nasal mucosal tissue.

Key words: allergic rhinitis, IgE, immunohistochemistry, mast cell, nasal mucosa.

INTRODUCTION

IgE is the primary class of antibody involved in the initiation of immediate allergic responses.¹ Allergen-specific and non-specific IgE bind to high-affinity type I Fcε receptors (FcεRI) on the cell membrane of mast cells.¹ When mast cells differentiate and proliferate from the mast cell precursor, they express FcεRI on the cell membrane.^{2,3} If there is IgE in the tissue where mast cells exist, IgE can

bind to FcεRI on the cells. We have previously confirmed, by immunohistochemical electron microscopy, that mast cells in allergic nasal mucosa have IgE bound to the surface.⁴

If cross-linking of polyvalent antigens (allergens) to two allergen-specific IgE bound to FcεRI occurs on mast cells, preformed and newly synthesized proinflammatory mediators, including cytokines, are released.¹ The cross-linked allergen-IgE/FcεRI complex does not remain on the cell surface but is, rather, internalized, entering the cytoplasm.⁵ The number of FcεRI on mast cells varies, depending on the immunologic status of the animal. Pawankar *et al.*⁶ have reported the FcεRI α chain of mast cells isolated from whole nasal mucosa to be more concentrated in allergic rhinitis mucosa than in non-allergic rhinitis mucosa. Moreover, the number of FcεRI α chains on mast cells of nasal mucosa correlated with serum IgE concentration.⁷

Allergic reactivity of mast cells is induced by interaction between IgE bound to FcεRI and allergen. Activation of mast cells may depend on the quantity of IgE on the cell surface and the amount of inhaled allergen. Nasal allergic symptoms are triggered by activation of mast cells in the allergic nasal mucosa. However, in the nasal mucosa of perennial allergic rhinitis, mast cells differ between the epithelial layer and the deep lamina propria with respect to the size of cells,⁸ the content of neural proteases (tryptase and chymase)⁹ and the ability to release histamine in response to sodium cromoglycate.¹⁰ More degranulation of mast cells in the allergic nasal mucosa has been observed in the epithelial layer than in the deep layer of the lamina propria by electron microscopy.¹¹ It is important to determine whether mast cells have the same quantity of IgE on the cell surface in different areas of the allergic nasal mucosa.

We stained IgE and mast cells from the nasal mucosa of patients with allergic and non-allergic rhinitis using anti-IgE and antitryptase antibodies. We measured the

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Received 24 July 2000. Accepted for publication 2 November 2000.

quantity of IgE on mast cells in three zones of allergic and non-allergic nasal mucosa, namely the epithelial layer, the subepithelial layer and the deep layer of the lamina propria.

METHODS

Subjects

All patients needed surgical treatment, namely turbinectomy, for their nasal symptoms. Twenty-six patients, 14 with allergic rhinitis (mean age 28 years; range 12–42 years) and 12 with non-allergic nasal disease (mean age, 28 years; range 15–39 years) were studied. The criteria for a diagnosis of allergic rhinitis caused by house dust mites were characteristic perennial allergic symptoms, such as repeated attacks of sneezing and watery rhinorrhea, eosinophilia in the nasal smear and a positive capsulated hydrophilic carrier polymer–radioallergosorbent test (CAP-RAST) to housedust 1 and *Dermatophagoides farinae*. A nasal provocation test with paper discs of housedust (Torii Co. Ltd, Tokyo, Japan) was positive. Patients with non-allergic disease, such as chronic rhinitis and hypertrophic rhinitis, had no attacks of sneezing and watery rhinorrhea and no eosinophilia in the nasal smear. These patients did not have a positive CAP-RAST to housedust, *Dermatophagoides*, pollens (birch and orchard grass) or mugwort.

Specimen preparation

The obtained medial side of the nasal mucosa of the inferior turbinate, 10–20 mm from the anterior edge, was sectioned, fixed with 3.5% neutral buffered formalin for 1 day and embedded in paraffin. Sections, 3 µm, were obtained and mounted on aminopropyltriethoxysilane-coated slides.

Antibodies for immunohistochemistry

Rabbit polyclonal antihuman IgE antibody (DAKO A/S, Glostrup, Denmark) and murine monoclonal antihuman mast cell tryptase antibody (Chemicon International Inc., Temecula, CA, USA) were used as primary antibodies. Anti-IgE antibody was diluted 1/500 in phosphate-buffered saline (PBS; 0.01 mol/L sodium phosphate, 0.3 mol/L NaCl, 0.01% Triton X-100, pH 7.2). Anti-tryptase antibody was diluted to a final concentration of 1 µg/mL in Tris-buffered saline (TBS; 0.05 mol/L Tris-HCl, 0.15 mol/L NaCl, 0.01% Triton X-100, pH 7.6). Normal

rabbit IgG (10 µg/mL; DAKO A/S) and mouse IgG1 (1 µg/mL; ICN Biomedicals Inc., Costa Mesa, CA, USA) were used as controls for negative staining.

Immunohistochemical staining

Paraffin sections were dewaxed in xylene, rehydrated and treated with 0.01 mol/L citric acid (pH 6.0) for 30 min at 90–99°C to remove the effects of formaldehyde fixation. After blocking endogenous peroxidase with 0.3% H₂O₂ in methanol for 20 min, sections were incubated with 10% heat-inactivated, normal pig serum in PBS and then incubated with anti-IgE antibody or negative control antibody overnight (approximately 16 h) at 4°C. Sections were incubated for 1 h at room temperature (RT) in each of the following: (i) a 1 : 300 dilution of biotinylated swine antirabbit immunoglobulins (Ig; DAKO A/S) in PBS; and (ii) a 1 : 300 dilution of peroxidase-conjugated streptavidin (DAKO A/S) in PBS. IgE-positive cells were stained red by amino-9-ethylcarbazole containing H₂O₂. After washing with distilled water and TBS, a second staining procedure was performed on the same sections. Sections were incubated with 10% heat-inactivated normal goat serum in TBS and then incubated with anti-tryptase antibody or negative control antibody for 1 h at RT. Sections were incubated with goat antimouse Ig (DAKO A/S; diluted 1 : 50 in TBS) for 1 h at RT and then with alkaline phosphatase–antialkaline phosphatase, mouse monoclonal (DAKO A/S; diluted 1 : 50 in TBS) for 1 h at RT. Positive immunoreactivity was stained blue by 6 mg fast blue BB (Sigma Chemical Co., St Louis, MO, USA) containing 1 mg naphthol AS-MX phosphate (Sigma Chemical Co.) and 1 mmol/L levamisole in 10 mL of 0.1 mol/L Tris-HCl buffer (pH 8.5). At the end of each step, slides were washed in PBS or TBS.

Quantitation of IgE-staining

We classified the intensity of IgE-staining on mast cells that also displayed tryptase-positive staining into four grades as either strong, moderate, weak or negative (Fig. 1).

We examined three zones of the nasal mucosa: (i) the epithelial layer; (ii) the subepithelial layer, which is located between the basal membrane and the underlying 330 µm of tissue; and (iii) the deep layer of the lamina propria, which is located under the subepithelial layer. The number of mast cells observed in the subepithelial layer and deep layer of the lamina propria in each nasal mucosa, both allergic and non-allergic, was 150–250 cells and

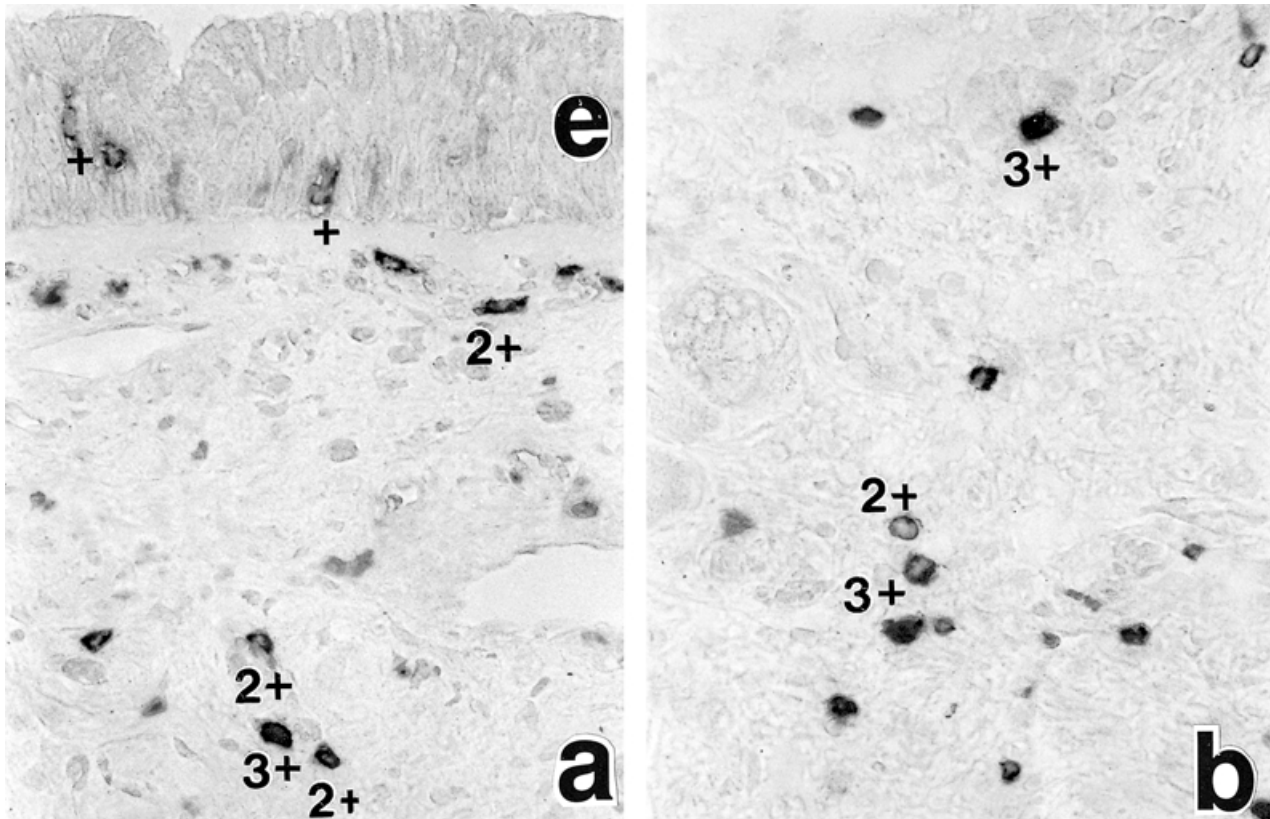


Fig. 1 IgE-positive mast cells in the allergic nasal mucosa visualized by double-labeling immunohistochemistry. (a) Epithelial layer and subepithelial layer. (b) Deep layer of the lamina propria. Red staining of original print corresponds to IgE immunoreactivity and blue staining corresponds to tryptase immunoreactivity. The intensity of IgE-staining on mast cells is classified into four grades as strong (3+), moderate (2+), weak (+) and negative (-). e, epithelium. Original magnification $\times 330$.

100–250 cells, respectively. The number of mast cells in the epithelial layer of each allergic nasal mucosa was 50–150 cells. A few mast cells were found in the epithelial layer of each non-allergic nasal mucosa.

We calculated the percentage of mast cells of each of the four grades indicated above in the epithelial layer, the subepithelial layer and the deep layer of the lamina propria of allergic nasal mucosa. In non-allergic nasal mucosa, mast cells in only the subepithelial layer and deep layer of the lamina propria were graded.

Statistical analysis

Statistical analysis of the percentage of each grade in each zone in allergic rhinitis specimens was performed by the Mann–Whitney *U*-test. Comparison of each grade in the subepithelial and deep layer of the lamina propria between allergic and non-allergic specimens was performed by the same method. $P < 0.05$ was considered statistically significant.

RESULTS

After the second immunostaining, most IgE-positive cells (red) also stained blue, indicating immunoreactivity with antitryptase antibody. When specimens were stained with control antibodies, no immunoreactivity was detected in either the first or second staining step. The quantity of IgE-bound mast cells in the three different regions of the allergic nasal mucosa is shown in Fig. 2. The mean percentage of mast cells displaying strong IgE-staining in the epithelial layer, subepithelial layer and deep layer of the lamina propria was 6, 18 and 27%, respectively. The mean percentage of strong positive IgE-stained mast cells in the epithelial layer and deep layer of the lamina propria differed significantly ($P < 0.01$). For moderately IgE-stained mast cells, the mean percentage was nearly equal in all three zones examined. Of the total number of mast cells, the percentage displaying weak IgE-staining in the epithelial layer, subepithelial layer and deep layer of the lamina propria was determined to be 58, 49 and

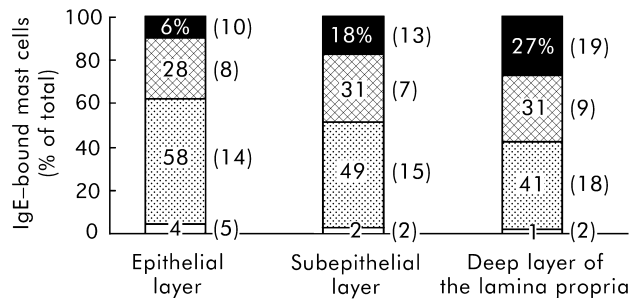


Fig. 2 The mean percentage of mast cells with strong (■), moderate (▣), weak (▤) or negative (□) IgE staining in three zones of the allergic nasal mucosa. The three zones are the epithelial layer, subepithelial layer and deep layer of the lamina propria. Parentheses indicate SD ($n = 14$).

41%, respectively. The mean percentage of weakly positive IgE-stained mast cells in the epithelial layer and deep layer of the lamina propria differed significantly ($P < 0.01$). IgE-negative tryptase-positive cells were detected at 1–4%.

The percentages of mast cells displaying IgE-staining in the subepithelial layer and deep layer of the lamina propria of allergic and non-allergic nasal mucosa are shown in Fig. 3. Because a few mast cells were detected in each epithelial layer of non-allergic mucosa (mean ($\pm$ SD) number of cells 4.6 ± 5.4), epithelial layers of allergic and non-allergic nasal mucosa were not compared. The quantity of mast cells displaying strong and moderate IgE-staining in the allergic nasal mucosa was significantly higher than that observed in non-allergic nasal mucosa ($P < 0.01$). Conversely, the quantity of mast cells displaying weak IgE-staining in the allergic nasal mucosa was significantly less than in non-allergic mucosa ($P < 0.05$).

A few tryptase-negative IgE-positive cells were observed in the nasal mucosa. The number of these cells was greater in allergic mucosa than in non-allergic mucosa.

DISCUSSION

IgE exists in both allergic nasal mucosal tissue and nasal secretion.^{12,13} We have reported detecting IgE immunoreactivity on all mast cells in the nasal mucosa of three allergic rhinitis patients by immunohistochemical electron microscopy, observing more than 30 mast cells.⁴ Several investigators have detected many IgE-positive cells in the nasal mucosa.^{14–16} Whether these IgE-positive cells were mast cells or another type of cell remains unknown. Cell types other than mast cells also have IgE present on the

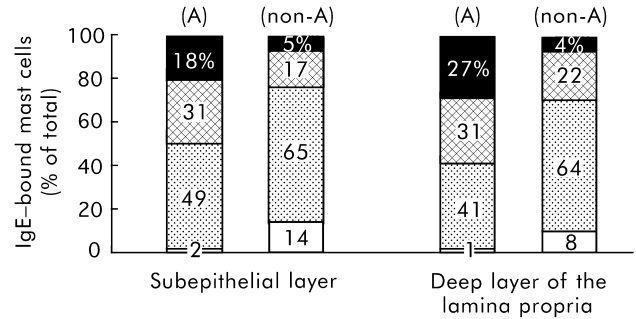


Fig. 3 Comparison of the percentage of mast cells having a strong (■), moderate (▣), weak (▤) or negative (□) grade of IgE staining in the lamina propria of allergic (A; $n = 14$) and non-allergic (non-A; $n = 12$) nasal mucosa. The percentage of IgE staining on mast cells in the epithelial layer of allergic and non-allergic nasal mucosa was not compared, because only a few mast cells were observed in each epithelial layer of the non-allergic mucosa.

surface of the cell membrane.¹ For example, basophils also express FcεRI¹ and exist in the allergic nasal mucosa.⁴ In the present study, we stained both IgE and tryptase using a double-labeling immunohistochemistry method and confirmed that IgE-positive cells were mast cells.

Our electron microscopic observations reported earlier indicated that, in the allergic nasal mucosa, degranulated mast cells were more abundant in the surface mucosa than in the deeper layers.¹¹ The intensity of IgE staining of mast cells in the allergic nasal mucosa did not correspond with the degranulation of mast cells.

There are two possible reasons why the quantity of mast cells having bound IgE on the cell surface varies among allergic nasal mucosal layers. First, the quantity of IgE present on mast cells in the different mucosal layers may depend on the concentration of FcεRI on the mast cell surface. For example, the mast cells in the deep layer of lamina propria are larger than cells in the epithelial layer.⁸ Due to the greater surface area of the large mast cell, many FcεRI may be present on the cell membrane. A precedent for this possibility has been reported, comparing large peritoneal mast cells with small intestinal mast cells in rat.¹⁷ If the IgE concentration in all areas of the nasal mucosa is the same, the large mast cells may have much more IgE than smaller mast cells. Second, concentrations of IgE vary in the different nasal mucosal layers. It has been proposed that the sites for production of allergen-specific and non-specific IgE are the regional lymphoid tissues, such as cervical lymph nodes, adenoids, tonsils or nasal mucosa.^{13–15,18,19} It is likely that IgE produced in regional lymphoid tissue is transported via

the circulatory system to vessels in the lamina propria of the nasal mucosa. In contrast, many T cells, B cells, mast cells and plasma cells are concentrated in the nasal lamina propria, so this region may also be a primary site of IgE production. IgE may then spread into all other areas within the nasal mucosa. Based on the assumption presented here, we speculate that the tissue IgE concentration is higher in the lamina propria than in the epithelial layer and, consequently, more IgE binds to mast cells localized in the lamina propria.

The mast cells in the allergic nasal lamina propria displayed a higher intensity of IgE-staining than cells in the non-allergic nasal lamina propria. The serum IgE concentration is higher in patients with allergic rhinitis than in non-allergic patients.²⁰ We speculate that the IgE level in nasal mucosal tissue may correlate with the serum IgE concentration and, consequently, more IgE binds to mast cells in allergic nasal mucosa than in non-allergic nasal mucosa.

A few IgE-negative mast cells were observed in the nasal mucosa. Mast cells without FcεRI have been detected by flow cytometric analysis.⁶ However, the absence of IgE staining on mast cells may be due to the detection limit of the staining technique and does not necessarily indicate that no IgE is present on the cell surface.

A few IgE-positive tryptase-negative cells were also observed. In an earlier study,⁴ we reported IgE-staining on the surface of basophils and in the cytoplasm of plasma cells. Moreover, FcεRI have also been detected in macrophages and eosinophils in allergen-induced rhinitic nasal mucosa.²¹ Therefore, the few IgE-positive tryptase-negative cells observed here may be immune cells other than mast cells.

In conclusion, our results suggest that mast cells in different regions of allergic nasal mucosa have different quantities of IgE on the cell surface. More IgE molecules were bound to the surface to mast cells in allergic nasal mucosa than in non-allergic nasal mucosa. Allergen-specific IgE have been detected on mast cells in the allergic nasal mucosa.²² Therefore, future studies should be directed towards correlating the quantity of allergen-specific IgE on the mast cell surface, mast cell activation and onset of allergic nasal symptoms.

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