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Nasal mast cells in experimentally induced allergic rhinitis in guinea-pigs

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Summary

The kinetics of mast cell accumulation in the nasal mucosa during allergen exposure were investigated in the animal model of experimentally induced allergic rhinitis. Guinea-pigs were divided into an unsensitized control group and five sensitized groups each containing five animals. Five sensitized groups were immunized intraperitoneally with ovalbumin, followed by intranasal administration of ovalbumin in each of four groups for 1, 2, 3 and 4 weeks, respectively. There were significant increases in the number of mast cells in the epithelium after 2 and 3 weeks in the groups receiving intranasal ovalbumin. No significant changes were detected in the lamina propria and the total number of mast cells. In the lamina propria, the distance between a mast cell and the basement membrane was significantly decreased in the groups after 2, 3 and 4 weeks of intranasal administration. These findings suggest that an infiltration of mast cells from the lamina propria to the epithelium occurred after 2 weeks of intranasal administration of allergen. An electron microscopic study showed no mast cells at the luminal surface of the epithelium and the majority of mast cells lying around the basal cells. This finding suggests that the allergen must penetrate into the epithelium in order to interact with mast cells in guinea-pig nose.

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Introduction

It is now generally accepted that interaction between allergen and IgE antibodies takes place on the surface of both mast cells and basophils resulting in the release of histamine and other chemical mediators, which are responsible for the symptoms of nasal allergy and asthma. Previous studies on the morphology and biology of basophilic cells in the nasal mucosa have indicated that most of these cells are mast cells [1,2]. A study in mice has indicated that mast cell precursors originate in the bone marrow and are carried by the blood to their final tissue of deposition, where they mature into recognizable mast cells [3]. Although an increase of mast cells in nasal mucosal epithelium has been reported in patients with allergic rhinitis after allergen provocation [4] and during the pollen season [5,6], the exact mechanisms of the increase of mast cells in mucosal epithelium are so far less well understood. Only Enerbäck *et al.* [5] have suggested intra-epithelial migration of mast cells from the subepithelial connective tissue stroma as a part of the mucosal response to seasonal allergen exposure.

The purpose of the present study was to examine the kinetics of mast cells in the nasal mucosa by using animal models of experimentally induced allergic rhinitis. The distribution of mast cells in the mucosal epithelium, which has rarely been reported, has also been studied.

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Materials and methods

Animal treatment

Male Hartlay guinea-pigs, weighing 300 g at the beginning of the experiment, were used. Animals were divided into an unsensitized control (C) group and five sensitized groups containing five animals each. The sensitized groups were immunized by an intraperitoneal injection of 0·02 mg ovalbumin (Nakarai Pharm, Kyoto, Japan) dissolved in 1 ml of 0·9% NaCl solution containing 10 mg Al $(OH)_3$ gel. Booster injections of the same antigen–Al $(OH)_3$ mixture were given intraperitoneally every 2 weeks five times after the initial immunization. One sensitized

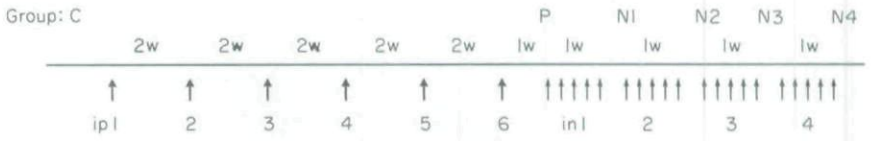

Fig. 1. Experimental protocol. ip. Intraperitoneal sensitization; in. Intranasal allergen challenge.

group was immunized only intraperitoneally (group P). On five consecutive days from the second week after the last peritoneal immunization, the other four groups inhaled a 0·01% ovalbumin solution generated by ultrasonic nebulization (NE-U11B, OMRON, Tokyo, Japan) which delivered 0·1 mg/min for 3 min. The nebulization was not performed on the next 2 days. The four groups (N1, N2, N3 and N4) received the ovalbumin intranasally for 1 to 4 weeks, respectively (Fig. 1).

The animals were anaesthetized intraperitoneally with sodium pentobarbital (Abbott Lab., North Chicago, USA). Blood samples were obtained and the serum was used for the passive cutaneous anaphylaxis (PCA) reaction. The animals were decapitated and the nasal septal mucosae were removed.

Titration of antibodies

Titres of IgE and IgG1 antibodies of pooled sera from each group were determined by PCA reactions [7]. In brief, shaved guinea-pigs were injected intradermally with 0·1 ml of serial dilutions of antisera. For determination of IgG1, sera were treated at 56°C for 30 min. The animals were challenged by an intravenous injection of 500 μg of ovalbumin in 1 ml of 0·5% Evans blue 8 days (for IgE) or 6 h (for IgG1) after the intradermal injection. Blue spots larger than 5 mm in diameter were considered as positive PCA reactions. The PCA titres are expressed as the reciprocal of the greatest dilution which produced a positive reaction.

Preparation of the specimens

The nasal mucosae were fixed with 2·5% glutaraldehyde in 0·1 mol/l phosphate buffer for 2 h at 4°C. They were further fixed in 1% osmium tetroxide for 1 h, dehydrated in a series of graded ethanols and embedded in Quetol 812 (Nisshin EM, Tokyo, Japan) followed by polymerization at 37°C for 12 h and at 60°C for 24 h.

Light microscopic observations

Serial sections were made for light and electron microscopy. Mast cells observed under a light microscope were confirmed by electron microscopy. For light microscopic observations, semi-thin (0·5 μm) sections were stained with 1% toluidine blue solution (pH 1·0). The numbers of mast cells in both the mucosal epithelium and in the lamina propria were counted under a light microscope. The area of the tissue section was measured by an image analyser (SP-500, Olympus, Tokyo, Japan) in combination with a light microscope. The mast cell number was expressed as the number of cells per cubic millimeter calculated by Lange's method [8]. The distance between the mast cells and the basement membrane was determined by measuring the distance from the centre of a nucleus of a mast cell to the basement membrane (Fig. 2). The distance was measured in all the mast cells examined in this study.

Electron microscopic observations

Ultra-thin (about 80 nm) sections were contrasted with uranyl acetate and lead citrate. These sections were observed using a H-800 electron microscope (Hitachi, Tokyo, Japan). Mast cells were identified by the morphological criteria reported by Dvorak *et al.* [9]. The position of mast cells in the epithelium was examined. The numbers of the mast cells examined were 33, 19, 34, 47, 43 and 32 cells in groups C, P, N1, N2, N3 and N4, respectively. The extent of degranulation of each mast cell was calculated by evaluation of cytoplasmic granules. To evaluate the degranulated granule the following criteria were used: swelling or lower electron density of the area enclosed by the perigranular membrane; lower electron density of granular substance with fibrillar, reticular and other changes; remnants of granular substance; the disappearance of granular substance; vacuolation; fusion of the peripherally located perigranular and plasma membrane [10]. A granule satisfying any of these criteria was regarded as a degranulated granule. The extent of degranulation was defined as the percentage of all degranulated granules to the granules in a cell. We calculated the extent of degranulation in all the mast cells examined in this study. Examples of the extent of degranulation are shown in Fig. 3.

Statistical analyses

The Wilcoxon's rank-sum test was used to compare the differences between groups. As a wide variation in the number of mast cells was observed among the guinea-pigs, the mean value was obtained from each animal. The values stated within the text are expressed as the mean $\pm$ SD. A *P*-value of less than 0·05 was considered significant.

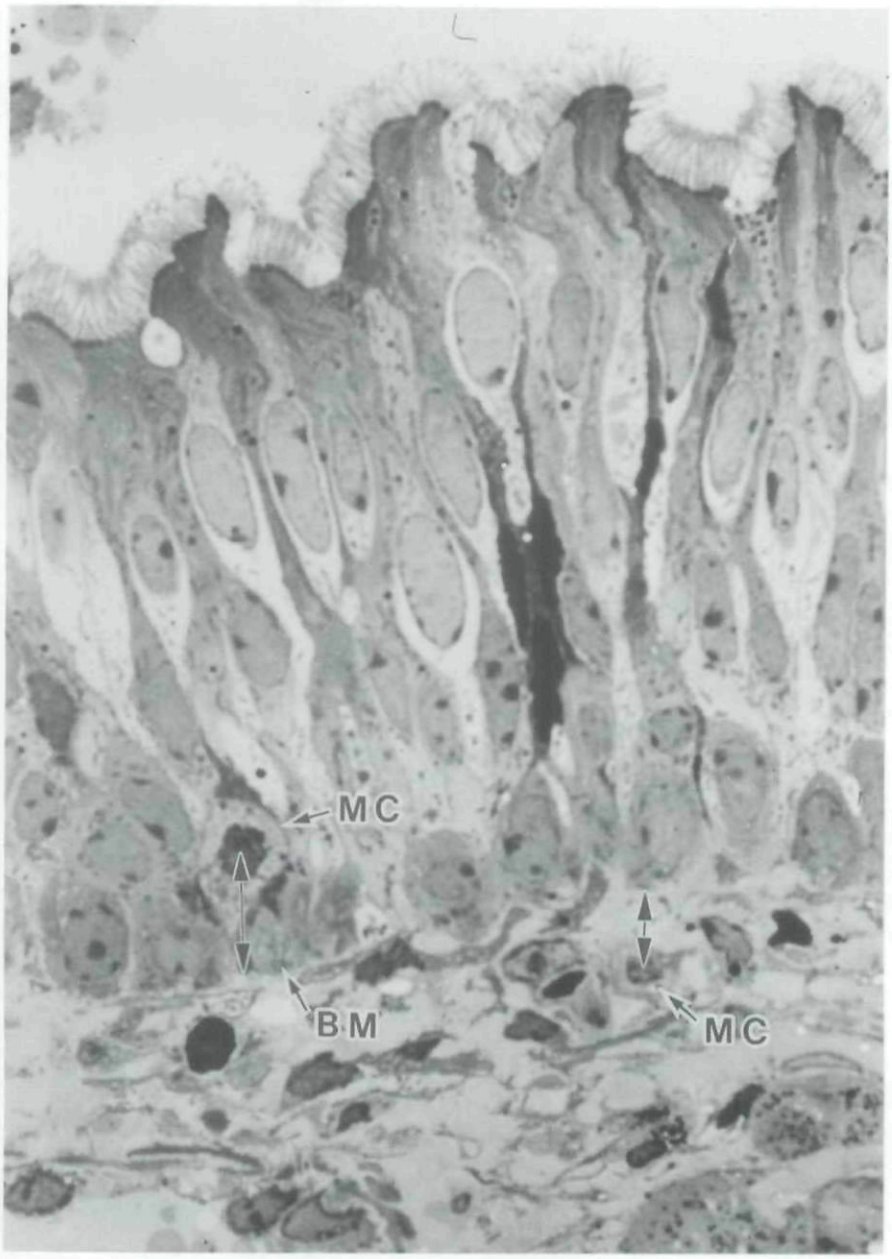

Fig. 2. The distance between each mast cell and the basement membrane. The arrows indicate the distance from the centre of the nucleus to the basement membrane. MC. Mast cell. BM. Basement membrane. × 1000.

Results

Passive cutaneous anaphylaxis

The 8-d PCA titres of group C were less than 8. Those of group P were between 100 and 400, and of groups N1 to N4, between 400 and 1600. In the 6-h PCA test for IgG1, positive reactions of 400 were noted in groups P and N, but not in group C.

Number of mast cells

Table 1 shows the number of mast cells per cubic millimeter in each group. The number of cells in the epithelium in group N2 was significantly higher than that in group N1. The cell population in group N3 was also significantly higher than that in group C and N1. No significant differences in the lamina propria and in the total number of cells were observed between groups.

Distance between mast cells and the basement membrane

Table 2 shows the mean distance between mast cells and the basement membrane in each group. In the epithelium, although there were statistical differences in the distance between groups N1 and N2, N2 and N3, and N3 and N4,

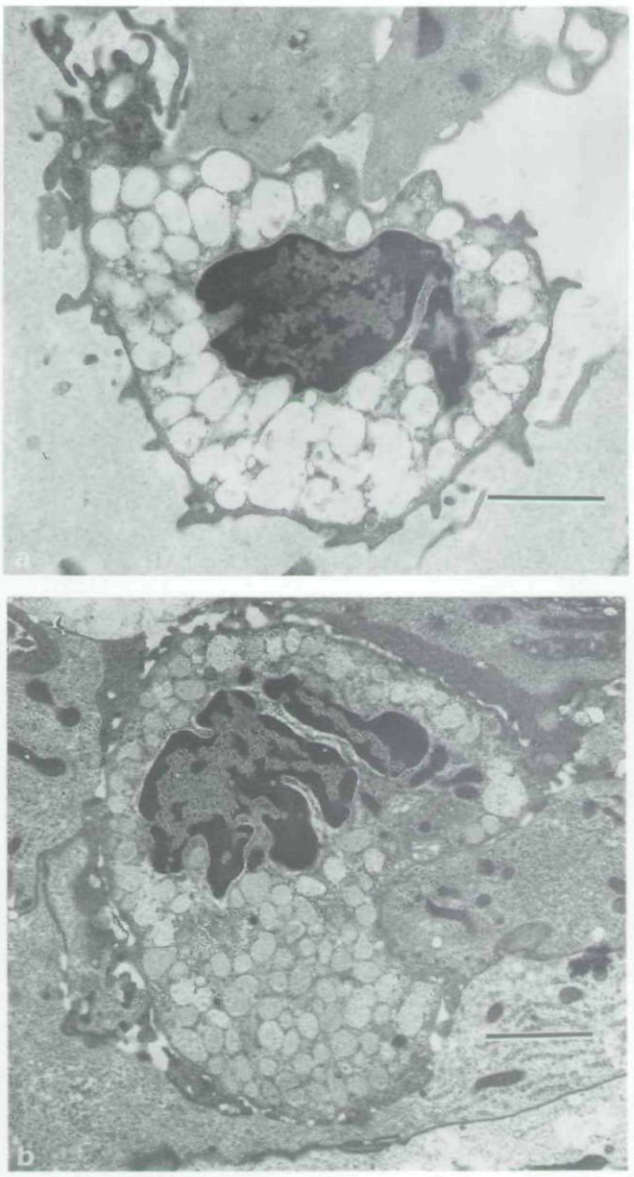

Fig. 3. Examples of the extent of mast cell degranulation. (a) A mast cell in group N4 showing complete (100%) degranulation. (b) A mast cell in group C with some intact granules. The extent of degranulation was calculated to be 76·1%. Bar = 2 μm.

no dominant trend was observed. In the lamina propria, the distance in groups N2, N3 and N4 was significantly reduced compared with that in group N1.

Extent of degranulation of mast cells

The extent of degranulation of mast cells in the epithelium was high even in group C, the mean values being more than 95% in all groups. Mast cells in the lamina propria of groups C and P were relatively intact compared with groups N1–4. The extent of degranulation in groups N1 and N4 was significantly higher than in group P. Mast cell degranulation in group N1 was also significantly higher than that of group C (Table 3).

Distribution of mast cells in the mucosal epithelium

With respect to the position of mast cells in the mucosal epithelium, no remarkable differences in the distribution of the cells were observed between groups. The results obtained from examination of a total of 208 mast cells showed that almost all the cells were observed in the vicinity of the basal cells and there were no mast cells in the luminal portion of the epithelium.

Discussion

In the present study, guinea-pigs were sensitized intraperitoneally with ovalbumin absorbed in $Ag(OH)_3$, and then intranasally exposed to aerosolized ovalbumin. These animals showed both positive unheated 8-d (IgE) and heated 6-h (IgG1) PCA reactions with high titres. Moreover, an increase in the mast cell population of mucosal epithelium after aerosolized allergen challenge is well correlated to the findings in humans reported by Pipkorn *et al.* [11], who showed mast cells starting to appear in the epithelium after 4–5 d of pollen exposure. Thus, our animal models could be useful for studying the kinetics of mast cell accumulation during nasal allergen exposure.

Our morphological study shows that the guinea-pig mast cells differ considerably from the human mast cells in nasal mucosa. Although Okuda, Kawabori and colleagues [2,12] observed many mast cells in the deep layer of lamina propria in human nasal mucosa, there are no mast cells in the deep layer in guinea pigs. Electron microscopical findings of mast cell granules in man showed them to be dense, multilamellar, scroll and reticular [13]. In contrast to human mast cells, the granules of guinea-pig mast cells appeared to be coarsely or finely granular, and sometimes contained scrolls or lamellar arrays, but rarely seemed homogeneous and dense.

In this study we investigated changes in the number of mast cells in the nasal mucosa during the intraperitoneal sensitization and intranasal administration of the allergen, and demonstrated significant increases in mast cell numbers in the mucosal epithelium between 2 and 3 weeks after intranasal administration. Changes in the numbers of mast cells in the lamina propria and in the total number in the mucosa were below the number of significance. Our results in this animal study are well correlated to those in the human study reported by Enerbäck, Pipkorn and colleagues [5,11]. They reported that although the total

Table 1. Mast cell numbers per cubic millimeter

	Group					
	C	P	N1	N2	N3	N4
Epithelium	2012±896	1862±1299	1450±920	3456±1341*	3792±1605*†	2724±1036
Lamina propria	335±567	1436±1444	820±853	1504±1477	572±763	464±511
Total mucosa	922±612	1373±685	982±592	1890±573	1522±769	1054±765

Values represent the mean ± SD ($n=5$) of mast cell numbers per cubic millimeter.
*$P<0.05$ *vs* N1.
†$P<0.05$ *vs* C.

Table 2. Distance between mast cells and the basement membrane (μm)

	Group					
	C	P	N1	N2	N3	N4
Epithelium	13·6±4·4	15·7±3·8	17·5±5·2	11·4±4·1*†	17·1±1·3	12·5±2·3†
Lamina propria	18·3±7·6	12·8±6·4	20·2±11·4	8·9±4·0*	7·0±1·4*	7·4±3·1*

Values represent the mean ± SD ($n=5$) of the distances between mast cells and the basement membrane.
*$P<0.05$ *vs* N1.
†$P<0.05$ *vs* N3.

Table 3. Extent of degranulation of the mast cells observed by electron microscope (%)

	Group					
	C	P	N1	N2	N3	N4
Epithelium	97·8±2·3	96·1±4·0	98·6±1·7	98·3±0·5	97·5±1·8	99·6±0·6
Lamina propria	88·6±7·9	81·7±12·3	99·4±0·6*†	93·3±1·2	95·9±5·2	96·6±1·6†

Values represent the mean ± SD ($n=5$) of the extent of degranulation of the mast cells.
*$P<0.05$ *vs* C.
†$P<0.05$ *vs* P.

number of mucosal mast cells in birch pollen-allergic patients during the pollen season did not change significantly with the onset of the pollen season, the percentage of intraepithelial mast cells did increase. They suggested a redistribution of mast cells into the epithelium by allergen-stimulated migration. Our study may further support their suggestion by the measurement of the distance between each mast cell and the basement membrane under a light microscope. In our quantitative study, mast cells in the mucosal epithelium showed a tendency to approach neither the basement membrane nor the luminal surface in the course of intranasal administration of the allergen. On the other hand, mast cells in the lamina propria after 2, 3 and 4 weeks of intranasal administration were significantly closer to the basement membrane compared with those after 1 week of intranasal administration. Moreover, a mast cell migrating through the basement membrane was noted under the electron micro-

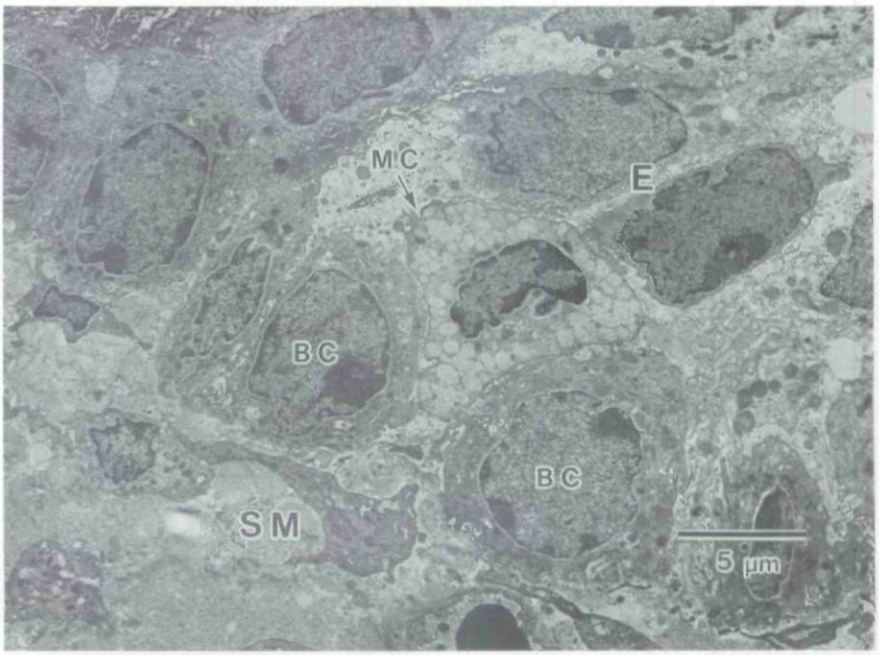

Fig. 4. A mast cell (MC) migrating into the epithelium. A part of the cell is seen between the two basal cells (BC). The specimen was obtained from group N2. E. Epithelium. SM. submucosa.

scope (Fig. 4). Our findings strongly suggest that a migration of mast cells from the lamina propria to the mucosal epithelium could take place after 2 weeks of intranasal application of the allergen.

It has been generally accepted that an increase in epithelial mast cells provides cells on the luminal surfaces to interact with the allergen [6,14]. However, in this study both light and electron microscopic findings showed no mast cells on the luminal surface of the epithelium. Almost all of the mast cells in the epithelium were seen around the basal cells. These observations suggest that the habitat of guinea-pig mast cells in the epithelium is around the basal cells. The distribution site of human epithelial mast cells must be clarified in further experiments.

In our present study, mast cells in the epithelium showed a greater extent of degranulation even in the control group. This may be due to species-specific features in guinea-pigs. However, the extent of degranulation of mast cells in the lamina propria of intranasally allergen-challenged groups was higher than that of control and intraperitoneally sensitized groups. These results indicate that mast cells could react with allergens, even in the lamina propria, during allergen exposure. It is assumed that inhaled allergens may react initially with sensitized basophils or mast cells at or close to the nasal epithelium. The mediators released from these cells may then cause the opening of the intra-epithelial tight junctions, allowing penetration of allergen into the epithelium and lamina propria where further interaction with sensitized mast cells may occur [15,16]. Our electron microscopic findings strongly support this hypothesis, and also indicate the importance of penetration of allergen not only into the mucosal epithelium but also into the submucosa. Since Inagaki *et al.* [17] demonstrated that the tight junction of the nasal epithelium in man becomes leaky in allergic conditions, a similar interaction between the allergen and submucosal mast cells could take place even in humans.

References

1 Okuda M, Ohtsuka H, Kawabori S. Basophil leukocytes and mast cells in the nose. Eur J Respir Dis 1983; 64(suppl 128):7–14.

2 Okuda M, Sakaguchi Y, Suzuki F, Ohtsuka H, Kawabori S. Ultrastructural heterogeneity of the basophilic cells in the allergic nasal mucosa. Ann Allergy 1985; 54:152–7.

3 Kitamura Y, Kasugai T, Morimoto M *et al.* Development of mast cells and basophils: Usefulness of mutant mice and rats. In: Miyamoto T, Okuda M, eds. Progress in allergy and clinical immunology 2. Seattle: Hogrefe & Huber Publishers, 1992:477–84.

4 Okuda M, Senba O. Effects of beclomethasone dipropionate nasal spray on subjective and objective findings in perennial allergic rhinitis. Clin Otolaryngol 1980; 5:315–21.

5 Enerbäk L, Pipkorn U, Granerus G. Intraepithelial migration of mucosal mast cells in hay fever. Int Archs Allergy Appl Immunol 1986; 80:44–51.

6 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; 82:275–6.

7 Ovary Z, Kaplan B, Kojima S. Characteristic of guinea pig IgE. Int Archs Allergy Appl Immunol 1976; 51:416–28.

8 Lange S, Nygren H, Svennerholm AM, Holmgren J.

Antitoxic cholera immunity in mice: influence of antigen deposition on antitoxin-containing cells and protective immunity in different parts of the intestine. Infection Immunity 1980; 28:17–23.

9 Dvorak AM, Dvorak HF, Galli SJ. Ultrastructural criteria for identification of mast cells and basophils in humans, guinea pigs, and mice. Am Rev Respir Dis 1983; 128:S49–52.

10 Kawabori S, Nakamura A, Okude Y. The reactivity of mast cells in nasal mucosa: comparison between allergic and nonallergic disease. Auris Nasus Larynx (Tokyo) 1987; 14:77–86.

11 Pipkorn U, Karlsson G, Enerbäck L. The cellular response of the human allergic mucosa to natural allergen exposure. J Allergy Clin Immunol 1988; 82:1046–54.

12 Kawabori S, Okuda M, Unno T. Mast cells in allergic nasal epithelium and lamina propria before and after provocation. Clin Allergy 1983; 13:181–9.

13 Enerbäck L, Pipkorn U, Olofsson A. Intraepithelial migration of mucosal mast cells in hay fever: ultastructural observations. Int Archs Allergy Appl Immunol 1986; 81:289–97.

14 Howarth PH, Wilson S, Lau L, Rajakulasingam K. The nasal mast cell and rhinitis. Clin Exp Allergy 1991; 21 (suppl 2):3–8.

15 Mygind N. Mediators of nasal allergy. J Allergy Clin Immunol 1982; 70:149–59.

16 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; 78:637–45.

17 Inagaki M, Sakakura Y, Itoh H, Ukai K, Miyoshi Y. Macromolecular permeability of the tight junction of the human nasal mucosa. Rhinology 1985; 23:213–21.

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