

Histochemical and functional characteristics of metachromatic cells in the nasal epithelium in allergic rhinitis: Studies of nasal scrapings and their dispersed cells

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Background: In allergic rhinitis, metachromatic cells in the nasal epithelium increase in number and are thought to play an important role in nasal allergic manifestation.

Methods: To determine immunohistochemical and functional characteristics of the metachromatic cells, nasal scrapings and their dispersed cells from patients with perennial allergic rhinitis were studied.

Results: Eighty-three percent of all metachromatic cells in dispersed cell preparations were tryptase-positive mast cells (MC_T), 10% were tryptase-chymase-positive cells (MC_{TC}), and 7% were negative ($n = 10$). The mean histamine content per metachromatic cell was 1.9 ± 0.2 pg. The histamine content and histamine release from nasal surface scrapings of patients sensitized with mite antigen were strongly correlated with the level of serum IgE antibody for mite antigen. The net histamine release from nasal scrapings was antigen-dose-dependent ($1:2 \times 10^7$ to $1:2 \times 10^3$ dilution), and the antigen stimulated release of up to 17% of cell-associated histamine within 5 to 7 minutes. Histamine release from nasal scrapings induced by calcium ionophore A23187 was up to 21% of cell-associated histamine within 2 to 4 minutes, but no histamine release was stimulated by compound 48/80, substance P, or poly-L-lysine. Histamine release from nasal scrapings was inhibited 46% (10^{-5} mol/L) to 96% (10^{-4} mol/L) by quercetin and 58% (10^{-4} mol/L) to 72% (10^{-3} mol/L) by sodium cromoglycate.

Conclusions: These findings show the predominant characteristics of mast cells in the nasal epithelium in allergic rhinitis, and this information may be useful in relation to a therapeutic approach. (*J ALLERGY CLIN IMMUNOL* 1995;96:528-36.)

Key words: Allergic rhinitis, tryptase-positive mast cell, tryptase-chymase positive cell, histamine release, RAST, sodium cromoglycate

In respiratory allergic diseases, allergic rhinitis, and bronchial asthma, metachromatic cells are thought to be triggered to release mediators, leading to the tissue reaction.^{1,2} Of these metachromatic cells, mucosal surface mast cells, which can

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Abbreviations used

FRMCS:	Formalin-resistant mast cells
FSMCS:	formalin-sensitive mast cells
HEPES:	N-2-hydroxyethylpiperazin-N-2-ethane-sulfonic acid
HPLC:	High-performance liquid chromatography
MC_T :	Tryptase positive mast cell
MC_{TC} :	Tryptase-chymase-positive mast cells
PBS:	Phosphate-buffered saline
PN:	Protein nitrogen
SCG:	Sodium cromoglycate

increase in number in the epithelium of the nasal mucosa or bronchus, are thought to play an important role especially in the immediate response.³⁻⁸

The interaction between inhaled antigen and IgE antibody on the surface of metachromatic cells at the mucosal level leads to release of chemical mediators and airway disease.³⁻⁸

In human beings, two subpopulations of mast cells have been recognized with respect to formalin sensitivity or insensitivity.⁹⁻¹² Previously, we reported two histochemically different subpopulations of mast cells in the human nasal mucosa with formalin sensitivity similar to that of rat mast cell subpopulations.¹³⁻¹⁵ Most metachromatic cells in the epithelium lost metachromatic properties after formalin exposure, and the number increased in relation to the degree of nasal provocative reactions to allergen, with resulting nasal symptoms and pathologic changes relating to swollen nasal mucosa in allergic rhinitis.^{5, 13} Irani et al.^{16, 17} and Schwartz et al.¹⁸ demonstrated two subpopulation of mast cells on the basis of neutral protease content; tryptase-positive mast cells (MC_T) and tryptase-chymase-positive mast cells (MC_{TC}). Church et al.¹⁹ and Benyon et al.²⁰ reported different characteristics in comparisons of MC_T-rich lung cell and MC_{CT}-rich skin cell preparations.

For in vitro functional studies, a large number of mast cells is required to achieve reproducibility of histamine release or inhibitory effects.¹⁹⁻²⁴ We chose patients who had more than 30 ng of histamine in a scraping of the nasal epithelial layer and who had swollen mucosa and a high level of sensitivity, above a score of 3 as determined by CAP RAST (Pharmacia, Uppsala, Sweden), for *Dermatophagoides pteronyssinus*; and we developed a model of histamine release by using these scrapings. With this method the functional study of metachromatic cells in nasal epithelium could be accomplished.

METHODS

Subjects

One hundred twenty-three patients (73 male and 50 female subjects; age range, 8 to 38 years; mean age $\pm$ SEM, 16 ± 0.6 years), who were sensitized to only one of 13 inhaled antigens (house dust mite) were studied. They had mild to severe perennial nasal symptoms and CAP RAST score of 1 to 6 for *D. pteronyssinus*.²⁵ Informed consent was obtained in all cases. Ten of these patients were selected for measurement of histamine content, 10 were selected for immunohistochemically distinct subtyping in metachromatic cells dispersed from nasal scrapings, and 37 were selected for study of the relationship between CAP RAST in serum and net histamine release induced by antigen from nasal scrapings. The releasing agent calcium ionophore A23187, compound 48/80, and substance P were used for the

study of histamine release with dose-dependence for antigen and inhibition of histamine release by antiallergic agent in 66 patients who were sensitized to mite, had a CAP RAST score of more than 3 for mite (*D. pteronyssinus*), and had pale swollen nasal turbinate (more than 30 ng histamine per scraping).

Nasal scrapings

Epithelial specimens of the nasal inferior turbinate were obtained by gentle scraping (without inducing bleeding) with a small curette (Nagashima Co., Tokyo, Japan) until the specimen filled the cup of the curette. With this procedure, about 6 mg (wet volume) of the epithelial layer was obtained.

Cell dispersal of nasal scrapings

Five scrapings were taken from one site in the nose and dispersed as described previously²³ by incubation for 30 minutes with a magnetic stirring apparatus at 37° C, in 25 ml of Ca⁺⁺- and Mg⁺⁺-free Hanks' balanced salt solution containing 25 mmol/L N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES), 1.3×10^{-4} mol/L ethylenediaminetetraacetic acid as a disodium salt, pH 7.2 to 7.4, 285 ± 15 mOsmol/kg. After pipetting, cells in the supernatant were filtered with nylon gauze and washed twice with HEPES-Tyrode buffer (NaCl, 137 mmol/L; glucose, 5.6 mmol/L; KCl, 2.7 mmol/L; NaH₂PO₄, 0.4 mmol/L; HEPES, 10 mmol/L; and 0.1% bovine serum albumin, pH 7.4). Cells were suspended in 3 ml of HEPES-Tyrode buffer. Then the cell suspension was divided into three parts of 1 ml each. The first one was used as follows: the total cell count in suspension and the proportion of metachromatic cells in cyto-spin smear were determined, and the others were used for duplicate histamine measurements. The smear was stained by May-Grunwald Giemsa solution, and the number of metachromatic cells in 2000 cells was determined. The suspension was boiled at 90° C for 10 minutes and centrifuged for 5 minutes at 12,400 g. Histamine was measured by high-performance liquid chromatography (HPLC) post-column method.²⁶ In a previous study a correlation coefficient of 0.9999 was obtained in blind comparisons of the concentration and the measurement by HPLC in range of 0.5 to 5000 ng/ml of histamine. A minimum of 0.2 ng of histamine can be measured by this method.

Immunohistochemistry

From nasal scrapings of the other 10 patients, cell smears were made on glass slides by cyto-spin and fixed in Carnoy's fluid (60% methanol/30% chloroform/10% glacial acetic acid) for 10 minutes. After washing with distilled water, endogenous peroxidase in the specimen was blocked by addition of 3% peroxidase in phosphate-buffered saline (PBS) at pH 7.4 for 10 minutes. After washing with PBS (pH 7.4), the specimen was incubated with 10% heat-inactivated normal goat serum for 1 hour at room temperature to reduce the nonspecific staining.

Then the specimen was washed with PBS for 2 minutes at room temperature.

Mouse anti-human mast cell tryptase (10 µg/ml) and chymase (10 µg/ml) in PBS, including 10 mmol/L Tris HCl at pH 7.8 and 10% normal goat serum as negative control, were incubated separately on slides overnight at 4° C. Samples were washed with PBS for 2 minutes and incubated with a biotin-conjugated rabbit anti-mouse IgG (Histofine kit; Nichirei Co., Tokyo, Japan) for 2 hours at room temperature. Samples were then washed with PBS for 5 minutes and stained with a diaminobenzidine substrate kit (Nichirei Co.)

Histamine release

Nasal scrapings (6 mg) were taken from the nasal turbinate surface with a small curette and attached to a small square piece of filter paper (16 mm², No. 4; Toyo Roshi Co., Tokyo, Japan), which was moistened with HEPES-buffered solution, and put into cold HEPES-buffered Tyrode's solution containing 0.1% bovine serum albumin without Ca⁺⁺ and Mg⁺⁺ (composed of 137 mmol/L NaCl, 5.6 mmol/L glucose, 2.7 mmol/L KCl, 0.4 mmol/L NaH₂PO₄, and 10 mmol/L HEPES [pH 7.4]) in a small plastic tube. Each specimen was then added to 900 µl of another prewarmed HEPES-buffered Tyrode's solution without Ca⁺⁺ and Mg⁺⁺ and incubated for 20 minutes at 37° C. Then the specimen was placed in 300 µl of another prewarmed HEPES-buffered Tyrode's solution containing 0.1% bovine serum albumin with 1.8 mmol/L CaCl₂ and 0.5 mmol/L MgCl₂ together with mite antigen for 30 minutes or other secretagogues for 20 minutes of incubation at 37° C. The reaction was terminated with 600 µl of cold HEPES-buffered Tyrode's solution. The tube was centrifuged at 200 g for 3 minutes, and the supernatant (A) was removed and put into another tube. Nine hundred microliters of HEPES-buffered Tyrode's solution (B) was added to the tube with the specimen and paper disk at the bottom. The histamine contents of A and B were determined by HPLC.²⁶ Histamine release was expressed as a percentage of the total content in a specimen and calculated as: Histamine in A ÷ (Histamine in A + Residual histamine in specimen B) × 100.

Time-course study

For the study of kinetics of histamine release from the nasal surface, four nasal scrapings on a piece of filter paper were put in 2 ml of HEPES-buffered Tyrode's solution with 1:20,000 dilution of mite antigen or calcium ionophore A23187; and histamine in 100 µl of supernatant taken at each time from the tube was measured by HPLC.

Test drugs

Mite antigen (*D. pteronyssinus*) extract was a gift from Torii Pharmaceutical Co. (Tokyo, Japan). The extract was eluted with HEPES buffer solution at 440 µg PN/ml (1:10 dilution) and frozen in 100 µl aliquots in small

tubes. Sodium cromoglycate (SCG) was a gift from Fisons Pharmaceuticals Ltd. (Loughborough, England). Calcium ionophore A23187, compound 48/80, substance P, poly-L-lysine, and quercetin were purchased from Sigma Chemical Co. (St. Louis, Mo.). Compound 48/80 was dissolved in HEPES-buffered Tyrode's solution at the time of each experiment. SCG was dissolved in distilled water at 10⁻² mol/L at the time of each experiment. Ionophore and quercetin were dissolved in dimethyl sulfoxide to produce stock solutions of 10⁻² mol/L. Each solution of antigen, secretagogues, and inhibitors was diluted in buffer as required. Substance P was dissolved in 0.1N acetic acid to produce stock solutions of 10⁻³ µmol/L and neutralized with 0.1N NaOH just before use. The final concentration of dimethyl sulfoxide (≤1%) was tested and had no effect on the secretory response. SCG and quercetin were added 10 seconds and 10 minutes before antigen.

Mouse monoclonal antibody (IgG₁) for anti-human mast cell tryptase or chymase was purchased from Chemicon International, Inc. (Temecula, Calif.), and the staining kit for the streptavidin-biotin method of immunohistochemistry was purchased from Nichirei Co.

Statistical analysis

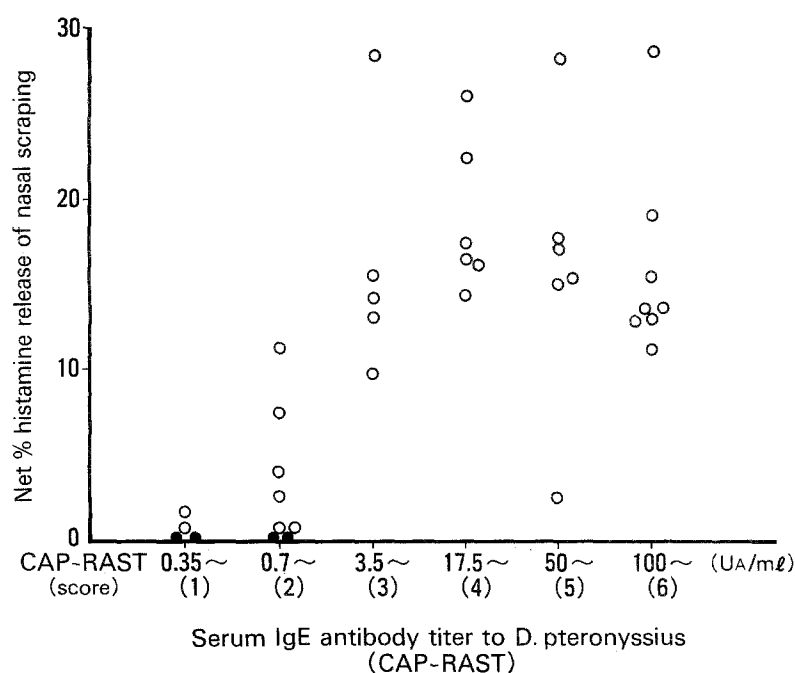
For comparisons of the percent histamine release and histamine content of nasal scrapings between groups with different scores of serum anti-mite IgE titer, F analysis was conducted to determine whether variances were equal. If this were true, *t* tests were used. If this were not true, the nonparametric Mann-Whitney U statistic was used.

RESULTS

Histamine content and immunohistochemical characteristics of metachromatic cells in dispersed epithelial cells

The proportion of metachromatic cells in cell suspension dispersed from nasal scrapings in 10 patients with allergic rhinitis who were selected at random ranged from 0.14% to 3.39% (mean, 1.1% ± 0.3%); most cells were epithelial cells. The number of metachromatic cells per 1 ml of dispersed cell suspension of nasal scrapings in the same patients ranged from 1068 to 97,626, and the histamine content of the cell suspensions was 2.4 to 252.0 ng/ml. The mean ± SEM of histamine content per metachromatic cell was 1.92 ± 0.25 pg (range, 0.91 to 3.69 pg).

In smears of dispersed cells from nasal scrapings of 10 patients with allergic rhinitis, mean and standard errors of the proportion of tryptase-positive only, tryptase-chymase-positive, and protease-negative (possibly basophil) metachromatic cells were: 82.8% ± 4.2%, 10.1% ± 2.0%, and 7.1% ± 3.5%, respectively.



Relationship between histamine content or histamine release of nasal scrapings and serum IgE antibody

On the other hand, the mean histamine content of four nasal scrapings taken from each patient was statistically increased ($p < 0.05$) in relation to the serum titer of anti-mite IgE from 0.35 to 3.5 U_A/ml, but there was no significant difference among groups with a score of 3 to 6 (Fig. 2). Histamine content in nasal scrapings was greater than 30 ng in 21 of 25 patients in groups with a score of 3 to 6 and in two of 12 patients in groups with a score of 1 to 2. These results suggested that the number of metachromatic cells in nasal scrapings of patients with nasal allergy and histamine release were correlated with the concentration of serum IgE antibody.

The following studies of histamine release by the mite antigen and releasing agents and the inhibition studies of the release by anti-allergic drugs were done in patients who had a score of more than 3 (3.5 U_A/ml) of serum IgE anti-mite antibody titer and more than 30 ng histamine content per scraping.

Antigen-induced histamine release

With six nasal scrapings taken from each of eight patients, mite antigen at a dilution of $1:2 \times 10^7$ (2.2×10^{-4} μg PN/ml) to $1:2 \times 10^3$ (2.2 μg PN/ml)

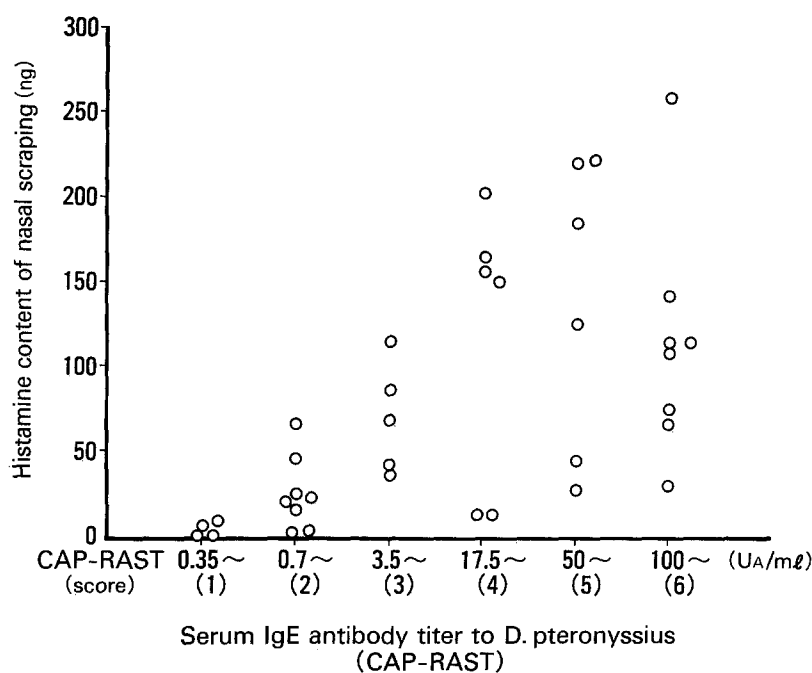


FIG. 2. Histamine content of nasal scrapings and score of serum IgE antibody to *D. pteronyssius*. Histamine content was increased ($p < 0.05$) according to the concentration of serum mite IgE antibody, but there was no significant difference among members of the group with a score of 3 to 6. Each circle indicates an individual case.

of mite extract) caused a concentration-related increase in histamine release from human nasal scrapings (Fig. 3, *A*). The means of net histamine release at dilutions of $1:2 \times 10^7$, $1:2 \times 10^6$, $1:2 \times 10^5$, $1:2 \times 10^4$, and $1:2 \times 10^3$ of mite concentrations were 1.3 ± 0.8 , 3.8 ± 0.8 , 9.2 ± 1.4 , 18.7 ± 2.0 , and 19.1 ± 2.0 , respectively. The net histamine release was increased in direct proportion to the concentration of the mite antigen, with a plateau at the dilution between $1:2 \times 10^4$ and $1:2 \times 10^3$. This $1:2 \times 10^4$ dilution was used to study the inhibition of histamine release from nasal scrapings by the drug (approximately 20%). In the same patients ($n = 8$) spontaneous histamine release from nasal scrapings was $7.5\% \pm 0.9\%$.

In six time-course studies (Fig. 3, *B*), histamine release from nasal scrapings induced by $1:2 \times 10^4$ dilution of mite antigen reached a plateau 5 to 7 minutes after challenge. About 30% of release occurred by 1 minute and 55% at 2.5 to 30 minutes. Spontaneous release was $8.5\% \pm 1.2\%$ at 30 minutes.

Histamine release by calcium ionophore A23187, compound 48/80, and poly-L-lysine

In nasal scrapings histamine release was induced by $0.5 \mu\text{mol/L}$ calcium ionophore A23187. However,

1 to $100 \mu\text{g/ml}$ compound 48/80 and 1 to $20 \mu\text{mol/L}$ substance P induced no histamine release. Poly-L-lysine at $10 \mu\text{mol/L}$ histamine release was $3.2\% \pm 2.2\%$ (Table I). Spontaneous histamine release from nasal scrapings of the same patients was $7.6\% \pm 0.6\%$ to $9.6\% \pm 0.8\%$. In six time-course studies (Fig. 4), histamine release induced by $1 \mu\text{mol/L}$ calcium ionophore A23187 reached a plateau 2 to 4 minutes after challenge. Spontaneous release was $8.2\% \pm 0.5\%$ at 30 minutes.

Inhibition of histamine release by SCG and quercetin

Four scrapings were taken from one site in the nasal cavity. In a control group, each scraping was incubated in a different tube with only HEPES buffer. In the inhibition study group of SCG or quercetin, first specimen was incubated with only HEPES buffer, and second with the mite antigen ($1:2 \times 10^4$ dilution); third and fourth specimens were preincubated with 10^{-4} mol/L and 10^{-3} mol/L SCG or 10^{-5} mol/L (final concentration) quercetin, respectively, before the antigen. In a control group the net histamine release values among second, third, and fourth trials were not statistically different. On the other hand, after addition of SCG, the net

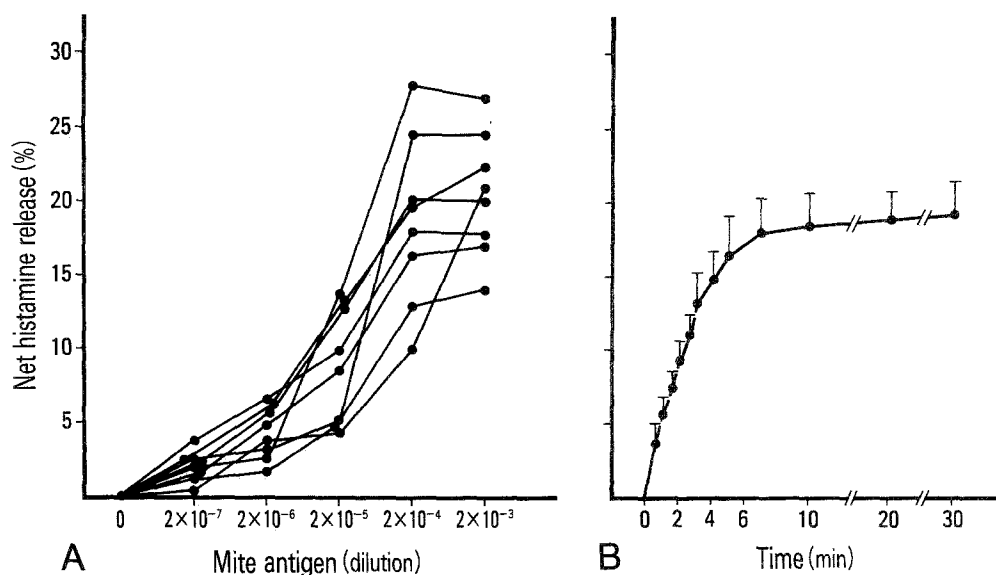


FIG. 3. Mite antigen-induced histamine release from nasal scrapings of eight patients with mite allergy with over than $3.5 \text{ U}_A/\text{ml}$ serum mite IgE antibody and 30 ng of histamine content in nasal scrapings. **A**, Antigen concentration-dependent release. **B**, Time course of histamine release induced by mite antigen $1:2 \times 10^4$ dilution, which includes $0.22 \text{ } \mu\text{g}$ PN/ml. Each point shows the mean $\pm$ SEM of six experiments.

histamine release values were $8.7\% \pm 2.6\%$ (10^{-4} mol/L) and $6.5\% \pm 2.9\%$ (10^{-3} mol/L) with statistical difference ($p < 0.01$) compared with $19.4\% \pm 1.9\%$ (only allergen). After addition of quercetin, these values were $10.7\% \pm 1.8\%$ (10^{-5} mol/L), $0.6\% \pm 1.4\%$ (10^{-4} mol/L), which were statistically less ($p < 0.05$, $p < 0.01$) than $20.2\% \pm 2.2\%$ in control specimens (Table II). The percent inhibitions of histamine release by SCG were 58% at 10^{-4} mol/L and 72% at 10^{-3} mol/L SCG and 45% at 10^{-5} mol/L and 96% at 10^{-4} mol/L quercetin. Spontaneous release was $8.2\% \pm 0.6\%$ to $10.4\% \pm 1.5\%$.

DISCUSSION

In nasal allergy, metachromatic cells of the nasal surface are increased in number and are important for nasal manifestations of disease.^{4, 5, 13} Of these metachromatic cells, basophils appear abundantly in nasal secretions and mast cells in nasal epithelium.^{13, 27, 28} Our results showed that more than 80% of all metachromatic cells of the human allergic nasal epithelium were tryptase-positive mast cells, which were immunohistochemically similar to human lung mast cells.¹⁸ The average histamine content per metachromatic cell in dispersed cells of nasal epithelial scrapings of 10 patients with nasal allergy was $1.9 \pm 0.2 \text{ pg}$ (0.9 to 3.7 pg). This is similar to human intestinal mast

TABLE I. Percent histamine secretion from nasal scrapings stimulated by secretagogues

Compound	1 $\mu\text{g/ml}$	10 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
48/80 (4)	$-1.5 \pm 0.9^*$	-0.1 ± 1.0	0.0 ± 0.6
A23187 (5)	0.1 $\mu\text{mol/L}$ 3.3 ± 4.5	0.5 $\mu\text{mol/L}$ 11.1 ± 2.2	1 $\mu\text{mol/L}$ 20.6 ± 0.8
Substance P (8)	1 $\mu\text{mol/L}$ -1.4 ± 1.6	10 $\mu\text{mol/L}$ -0.9 ± 1.3	20 $\mu\text{mol/L}$ 1.9 ± 1.7
Poly-L-lysine (6)	1 $\mu\text{mol/L}$ 0.8 ± 1.0	10 $\mu\text{mol/L}$ 0.9 ± 1.2	100 $\mu\text{mol/L}$ 3.2 ± 2.2

Numbers in parentheses indicate the number of specimens. Incubation time was 20 minutes for all secretagogues.

*Mean $\pm$ SEM (%).

cells^{23, 24} but lower than the histamine content in dispersed cells from the lungs and skin.^{20, 21} The histamine content of dispersed mast cells has been reported to be 1 to 2 pg in intestine,^{23, 24} 2.5 pg in cells of smallest diameter, 10 pg in the largest cells in the lungs,²¹ and 4.6 pg in the skin.²⁰ In spite of similar distributions of mast cell subpopulations, such differences of histamine content in metachromatic cells in nasal epithelium and lung mast cells might be due to contained basophils in the epithelium and also to the degree of degranulation or cell development in the affected nasal mucosa. As demonstrated in this study, the proportions of metachromatic cells in nasal epithelia of 10 pa-

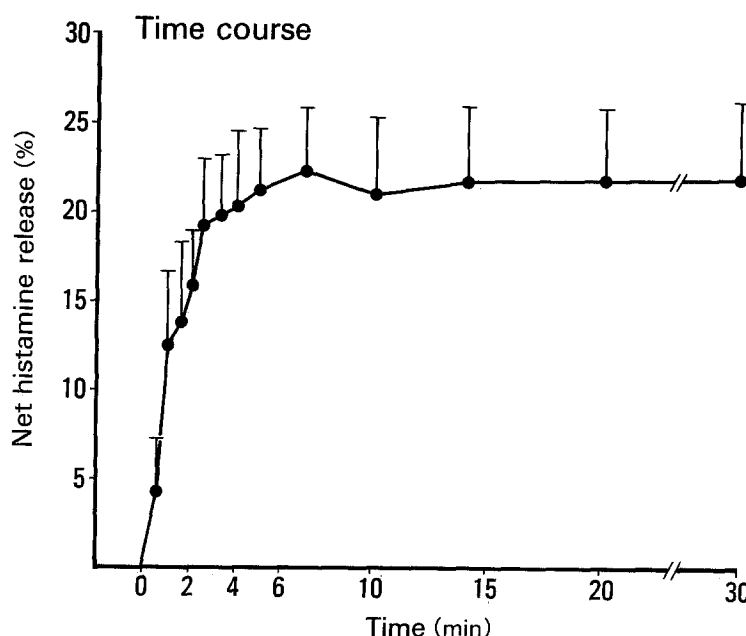


FIG. 4. Time course (~30 minutes) of histamine release induced by calcium ionophore A23187 $1 \mu\text{mol/L}$ from nasal scrapings. Each point shows the mean $\pm$ SEM of six experiments.

tients with allergic rhinitis ranged from 0.14% to 3.39% (mean, $1.1\% \pm 0.3\%$); most cells were epithelial cells. Immunohistochemical study of dispersed cells of the nasal epithelium showed that the mean proportions of MC_T , MC_{TC} , and protease negative cells (possibly basophils) ($n = 10$) were 83% (range, 58% to 100%), 10% (0% to 16%), and 7% (0% to 28%), respectively. Previously, we reported that more than 80% of surface nasal metachromatic cells were formalin-sensitive mast cells (FSMCS), and less than 5% were microscopically small with a segmented nucleus, and were considered to be basophils. In contrast, most of the cells in the deep area of the nasal mucosa were formalin-resistant mast cells (FRMCS).¹³ Kawabori and Kanai²⁹ showed that the distribution of MC_T and FSMCS, MC_{TC} and FRMCS tended to be similar in the nasal mucosa and that most mast cells in the epithelium were MC_T or FSMCS. Bentley et al.³⁰ mentioned that the number of MC_T in the nasal epithelia of the patients with seasonal allergic rhinitis increased during the allergy season.

Metachromatic cells in the nasal epithelium released histamine in antigen dose-dependent fashion. On the other hand, histamine release by nasal scrapings at the $1:2 \times 10^4$ dilution of the mite antigen was increased dependently with the concentration of the serum mite IgE antibody, and the release reached a plateau at a score of 3. And there was no significant difference among groups with

scores of 3 to 6 (range, 3.5 to greater than 100 U_A/ml) of CAP-RAST IgE measurement. These results might indicate that IgE receptors on metachromatic cells were fully occupied at an IgE antibody concentration of 3.5 U_A/ml in the tissue. This phenomenon is similar to the basophil response to antigen in individual patients, which is dependent on the serum IgE antibody level.³¹ The antigen-induced histamine release from nasal scrapings of eight patients with more than 3.5 U_A/ml of serum mite IgE antibody level revealed an antigen dose dependence. The mean spontaneous release was around 10%. However, net histamine release from nasal scrapings induced by antigen was 16% to 20%, which was similar to that induced by anti-IgE from dispersed mast cells of intestine^{23, 24} and greater than that in the skin.²⁰ On the other hand, in time-course studies, histamine release by antigen ($n = 8$) reached a plateau 5 to 7 minutes after challenge, which was similar to human skin mast cells as described previously.²⁰ These in vitro phenomena were considered to be similar to in vivo phenomena because the nasal responses of sneezing and nasal discharge, induced mainly by histamine, begin immediately and stop within 5 minutes after nasal antigen challenge.³²

More importantly, histamine contents in nasal scrapings, which might indicate the number of increased metachromatic cells, were in proportion to the serum IgE antibody level. In allergic rhinitis

TABLE II. Inhibition of antigen-induced histamine release by SCG and quercetin

Agent		Antigen	Antigen	Antigen
Control (<i>n</i> = 9)	% Net HR % Inhibition	17.5 ± 2.1	15.2 ± 0.5 −4.7 ± 3.3	16.2 ± 2.6 −6.5 ± 3.4
SCG (<i>n</i> = 8)	ND % Net HR % Inhibition	19.4 ± 1.9	10 ^{−4} mol/L 8.7 ± 2.6* 58.4 ± 10.4	10 ^{−3} mol/L 6.5 ± 2.9* 71.8 ± 10.4
Quercetin (<i>n</i> = 4)	ND % Net HR % Inhibition	20.2 ± 2.2	10 ^{−5} mol/L 10.7 ± 1.8† 45.7 ± 10.9	10 ^{−4} mol/L 0.6 ± 1.4* 96.3 ± 8.7

SCG was added 10 seconds and quercetin was added 20 minutes before addition of antigen. Scale indicates mean ± SEM (%) of histamine release or inhibition of histamine release. Numbers in parentheses indicate the number of specimens.

HR, Histamine release; ND, no drug added.

**p* < 0.01.

†*p* < 0.05.

the flux of mast cells of the epithelial layer of the nasal mucosa is caused by antigen exposure, and the resulting increase of nasal hypersensitivity is an important factor in nasal responses.^{5, 13, 33, 34}

The mean histamine content of nasal scrapings was increased in relation to the levels of serum IgE antibody and reached a plateau at 3.5 U_A/ml. However, as shown Fig. 2, the histamine content varied greatly among individual patients, even in those with a high concentration of serum IgE antibody (12.9 to 156 ng in score 4 and 25 to 267 ng in score 6). These differences in histamine content might be due to different numbers of metachromatic cells, which influence the nasal response.^{5, 13} These results indicate that nasal scrapings of patients with more than 3.5 U_A/ml of the serum mite antibody level and more than 30 ng of total histamine per scraping could be useful for functional studies of metachromatic cells at the nasal surface.

Histamine release from metachromatic cells of nasal epithelium by calcium ionophore A23187 was dose-dependent and reached to 20.6% at 1 μmol/L. In time-course studies, the histamine release reached a plateau at 2 to 4 minutes after challenge, which was faster than antigen-induced reactions. On the other hand, compound 48/80 at 1 to 100 μg/ml and substance P at 1 to 20 μmol/L did not lead to histamine release. Poly-L-lysine at 10 μmol/L induced release of a little histamine (3.2%), which was thought to be due to basophils and MC_{TC} included in nasal epithelium; both of these subtypes release histamine in response to poly-L-lysine.^{20, 35} SCG inhibited antigen-induced histamine release 58% at 10^{−4} mol/L and 72% at 10^{−3} mol/L, and quercetin inhibited it 45% at 10^{−5} mol/L and 96% at 10^{−4} mol/L. These results indicate that MC_T of metachromatic cells were

abundant in the nasal epithelium and functionally similar to lung mast cells, as reported by Church and Hiroi.³⁶ Irani et al.^{16, 17} and Schwartz et al.¹⁸ reported that 93% of mast cells dispersed from the lungs were MC_T and 99% of these cells from skin were MC_{TC}. Church et al.¹⁹ and Benyon et al.²⁰ reported that mast cells dispersed from human lungs released histamine in response to anti-IgE or calcium ionophore A23187 but not in response to compound 48/80, substance P, or poly-L-lysine; whereas human skin mast cells released histamine in response to compound 48/80, substance P, and poly-L-lysine. These findings suggest that epithelial metachromatic cells are similar to lung mast cells in human beings. Moreover, SCG inhibited antigen-induced histamine release from nasal epithelial metachromatic cells. The percentage of inhibition was more than 70% when nasal scrapings were used. Church and Hiroi³⁶ mentioned that the inhibitory action of SCG on antigen-induced histamine release was dependent on the dose of stimulating antigen. In our studies 1:2 × 10⁴ dilution of mite antigen was optimal. The antigen-induced histamine release from nasal scrapings was inhibited by quercetin, which inhibits mast cell and basophil release in rodents and human beings.^{37, 38}

The functional differences between metachromatic cells with abundant MC_T in the nasal epithelium and MC_{CT} in lamina propria of nasal mucosa remain to be determined. Such differences might be elucidated by future investigations.

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REFERENCES

- Ishizaka T, Ishizaka K. Activation of mast cells for mediator release through IgE receptor. *Prog Allergy* 1984;34:188-230.
- Zweiman B. Mast cells in human disease. *Clin Rev Allergy* 1983;1:417-26.
- Patterson R, Suzuko IM, Harris KE. The in vivo transfer of antigen-induced airway reactions by bronchial lumen cells. *J Clin Invest* 1978;62:519-24.
- Okuda M. Mechanisms in nasal allergy. *ORL Digest* 1977; 39:26-34.
- Ohtsuka H, Okuda M. Important factors in nasal manifestation of allergy. *Arch Otorhinolaryngol* 1981;233:227-35.
- Schleimer RP, Fox CC, Naclerio RM, et al. Role of human basophils and mast cells in the pathogenesis of allergic diseases. *J ALLERGY CLIN IMMUNOL* 1985;76:369-74.
- Kawabori S, Okuda M, Unno T, Nakamura A. Dynamics of mast cell degranulation in human allergic nasal epithelium after provocation with allergen. *Clin Allergy* 1985;15:509-15.
- Holgate ST, Hardy C, Robinson C, Agius RA, Howarth PH. The mast cell as a primary effector cell in the pathogenesis of asthma. *J ALLERGY CLIN IMMUNOL* 1986;77:274-82.
- Strobel S, Miller HRP, Ferguson A. Human intestinal mucosal mast cells: evaluation of fixation and staining techniques. *J Clin Pathol* 1981;34:851-8.
- Ruitenbergh EJ, Gustowska L, Elgersma A, Ruitenbergh HM. Effect of fixation on the light microscopical visualization of mast cells in the mucosa and connective tissue of the human duodenum. *Int Arch Allergy Appl Immunol* 1982;67:233-8.
- Enerback L. Mucosal mast cells in the rat and in man. *Int Arch Allergy Appl Immunol* 1987;82:249-55.
- Befus D, Goodacre R, Dyck N, Bienenstock J. Mast cell heterogeneity in man. I. Histologic studies of the intestine. *Int Arch Allergy Appl Immunol* 1985;76:232-6.
- Otsuka H, Denberg J, Dolovich J, et al. Heterogeneity of metachromatic cells in human nose: significance of mucosal mast cells. *J ALLERGY CLIN IMMUNOL* 1985;76:695-702.
- Otsuka H, Dolovich J, Befus D, Bienenstock J, Denburg J. Peripheral blood basophils, basophil progenitors, and nasal metachromatic cells in allergic rhinitis. *Am Rev Respir Dis* 1986;133:757-62.
- Otsuka H, Dolovich J, Richardson M, Bienenstock J, Denburg JA. Metachromatic cell progenitors and specific growth and differentiation factors in human nasal mucosa and polyps. *Am Rev Respir Dis* 1987;136:710-7.
- Irani AA, Schechter NM, Craig SS, DeBlois G, Schwartz LB. Two types of human mast cells that have distinct neutral protease composition. *Proc Natl Acad Sci USA* 1986;83:4464-8.
- Irani AA, Bradford TR, Kepley CL, Schechter NM, Schwartz LB. Detection of MC_T and MC_{TC} types of human mast cells by immunohistochemistry using new monoclonal anti-tryptase and anti-chymase antibodies. *J Histochem Cytochem* 1989;37:1509-15.
- Schwartz LB, Irani AA, Roller K, Castells MC, Schechter NM. Quantitation of histamine, tryptase, and chymase in dispersed human T and TC mast cells. *J Immunol* 1987; 138:2611-5.
- Church MK, Pao GJ-K, Holgate ST. Characterization of histamine secretion from mechanically dispersed human lung mast cells: effects of anti-IgE, calcium ionophore A23187, compound 48/80, and basic polypeptides. *J Immunol* 1982;129:2116-21.
- Benyon RC, Lowman MA, Church MK. Human skin mast cells: their dispersion, purification, and secretory characterization. *J Immunol* 1987;138:861-7.
- Schulman ES, Kagey-Sobotka A, MacGlashan DW Jr, Adkinson NF, Peters SP, Lichtenstein LM. Heterogeneity of human mast cells. *J Immunol* 1983;131:1936-41.
- Schleimer RP, MacGlashan DM Jr, Peters SP, Pinckard RN, Adkinson NF, Lichtenstein LM. Characterization of inflammatory mediator release from purified human lung mast cells. *Am Rev Respir Dis* 1986;133:614-7.
- Befus AD, Dyck N, Goodacre R, Bienenstock J. Mast cells from the human intestinal lamina propria: isolation, histochemical subtypes, and functional characterization. *J Immunol* 1987;138:2604-10.
- Rees PH, Hillier K, Church MK. The secretory characteristics of mast cells isolated from the human large intestinal mucosa and muscle. *Immunology* 1988;65:437-42.
- Ewan PW, Coote D. Evaluation of capsulated hydrophilic carrier polymer (the Immuno CAP) for measurement of specific IgE antibodies. *Allergy* 1990;45:22-9.
- Yamatodani A, Fukuda H, Wada H, Iwada T, Watanabe T. High-performance liquid chromatographic determination of plasma and brain histamine without previous purification of biological samples: cation-exchange chromatography coupled with post-column derivatization fluorometry. *J Chromatogr* 1985;344:115-23.
- Okuda M, Otsuka H. Basophilic cells in allergic nasal secretions. *Arch Otorhinolaryngol* 1977;214:283-9.
- Hastie R, Chir B, Heroy JH III, Levy DA. Basophil leukocytes and mast cells in human nasal secretions and scrapings studied by light microscopy. *Lab Invest* 1979;40:554-61.
- Kawabori S, Kanai N. Distribution of MC_T and MC_{TC} in nasal mucosae: Relationship with formalin sensitive mast cells and formalin resistant mast cells. *Jpn J Allergol* 1993;42:894-9.
- Bentley AM, Jacobson MR, Cumberworth V, et al. Immunohistology of the nasal mucosa in seasonal allergic rhinitis: increases in activated eosinophils and epithelial mast cells. *J ALLERGY CLIN IMMUNOL* 1992;89:877-83.
- Norman P. Correlation of RAST with in vivo and in vitro assay. In: Evans R III, ed. *Advances in diagnosis of allergy: RAST*. Proceedings of the First North American Conference on RAST. Miami: Medical Book Publishers, 1975:45-56.
- Clarke PS. The diagnosis of perennial rhinitis due to house dust mite (*Dermatophagoides pteronyssinus*) demonstrated by nasal provocation tests. *Ann Allergy* 1984;59:25-8.
- Enerback L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int Arch Allergy Appl Immunol* 1986;80:44-51.
- Martin TR, Takeishi T, Katz HR, Austen KF, Drazen JM, Galli SJ. Mast cell activation enhances airway responsiveness to methacholine in the mouse. *J Clin Invest* 1993;91:1176-82.
- Foreman JC, Lichtenstein LM. Induction of histamine secretion by polycations. *Biochim Biophys Acta* 1980;629: 587-603.
- Church MK, Hiroi J. Inhibition of IgE-dependent histamine release from human dispersed lung mast cells by anti-allergic drugs and salbutamol. *Br J Pharmacol* 1987;90:421-9.
- Pearce FL, Befus AD, Bienenstock J. Mucosal mast cells. III. Effect of quercetin and other flavonoids on antigen-induced histamine secretion from rat intestinal mast cells. *J ALLERGY CLIN IMMUNOL* 1984;73:819-23.
- Middleton E Jr, Drzewiecki G, Krishnarao D. Quercetin: an inhibitor of antigen-induced human basophil histamine release. *J Immunol* 1981;127:546-50.