American Journal of Allergy Asthma and Immunology Research

Research Article
Published: 25 Feb, 2022

Reactivity to Specific Antigen of Submucosa Mast Cells in Allergic Rhinitis was Very Low but Enhanced by Pretreated with Stem Cell Factor

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Abstract

Background: There are several papers that suggest the involvement of MCT increased in nasal surface of allergic rhinitis but not MCTC in nasal submucosa.

We studied whether MCTC in nasal submucosa could directly respond with specific antigen and stem cell factors (SCF) enhance the sensitivity of MCTC.

Methods: Using nasal subglandular submucosa and nasal surface scrapings of the inferior turbinate from patients sensitized solely to mite, we studied allergen induced histamine and leukotriene release, and whether allergen induced both mediators release from nasal subglandular submucosa was enhanced pretreatment with SCF.

Results: Histamine and leukotriene release from nasal scrapings were increased with mite allergen extract (from 2.2 ng PN/ml to 2.2 μg PN/ml). By contrast, those from fragments of nasal submucosa of same patients were very low under the same concentration of mite extract. However, when fragments of nasal submucosa were pre-treated with SCF, mite antigen 0.22 μg PN/ml induced–histamine and leukotriene release was significantly enhanced.

Conclusion: These findings suggested that MCTC in the submucosa mucosa were lower sensitive with specific antigen than MCT in nasal epithelium. It might be there they had a phenotype dependent on exogenouse SCF derived from epithelial cell to enhance allergen-induced release of histamine and leukotriene. Mast cells in nasal epithelium are constitutively more sensitive to allergen challenge, perhaps because of ongoing exposure to epithelial-derived SCF.

Keywords: Mast cell; Nasal submucosa; Nasal surface; Stem cell factor; Histamine release; Leukotriene release; Mite allergen

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Received Date: *02 Feb 2022*
Accepted Date: *18 Feb 2022*
Published Date: *25 Feb 2022*

Citation:
Otsuka H, Otsuka K, Okubo K. Reactivity to Specific Antigen of Submucosa Mast Cells in Allergic Rhinitis was Very Low but Enhanced by Pretreated with Stem Cell Factor. Am J Allergy Asthma Immunol Res. 2022; 1(1): 1003.

Introduction

Two subpopulations of human mast cells have been recognized with respect of formalin sensitivity of subsequent dye binding; Formalin-Sensitive Mast Cells (FSMC) and Formalin Resistant Mast Cells (FRMC) [1,2]. These two populations corresponded well to populations of mast cells distinguished by their content of serine proteases, namely Tryptase Mast Cells (MCT) and tryptase and Chymase Positive Mast Cells (MCTC) [3,4], respectively. In human nasal epithelium there are abundant FSMC or MCT (>80% of all metachromatic cells) which was called Mucosal Mast Cell (MMC), whereas in the nasal subgrandular layer >90% of all metachromatic cells are FRMC or MCTC called Connective Tissue Mast Cell (CTMC) [5,6].

MMCs in nasal surface were increased in numbers in perennial and seasonal allergic patients and were released allergen-induced histamine and leukotriene which were thought to play an important role [5-12]. Moreover, treatment of steroid for allergic rhinitis and allergic asthma reduced the number of FSMC or MCT in respiratory epithelium but did not reduce the number of FRMC or MCTC in submucosa despite the improvement in symptoms [13-15].

These findings supported the hypothesis that cells in the nasal surface trigger local allergic reactions [16-18]. However, this approach did not allow study of CTMCs in the submucosa.

Question is whether CTMCs in the nasal submucosa can release well these mediators by specific antigen as MMCs in the nasal surface.

Given abundant evidence that the microenvironment plays a critical role in the mast cell differentiation and phenotype, we postulated that CTMCs in the nasal submucosa might be different in their phenotype from MMCs in the nasal epithelium.

Previously, we and others have reported that nasal epithelial cells produced Stem Cell Factor (SCF) and that levels correlated with the number of mast cells in surface tissue of allergic rhinitis patients [19,20]. We suggested that SCF produced by the nasal epithelial cell enhanced mast cell proliferation and activation. Indeed, SCF has been showed in several studies to enhance IgE-dependent mediator release from mast cells and to alter the expression of selected genes in developing mast cell [21-26].

Accordingly, we compared histamine and leukotriene release from nasal epithelial scrapings with nasal submucosa derived from the same patients with allergic rhinitis. We investigated the effects of pretreatment with SCF on allergic-induced histamine and leukotriene release from nasal submucosa.

Patients

Eight patients (all male, average and standard error of age 19.5 ± 0.82) and 8 patients (all male, 21.2 ± 1.45) were selected for the comparative study allergen, dose-dependent histamine and leukotreien release respectively with extract of *Dermatophagoides pteronyssinus* (Dp) from nasal scrapings and nasal subglandular mucosa. Eight patients (7 male and 1 female, 22 ± 1.75) were studied for allergen-induced histamine and leukotriene release from nasal submucosa following pretreatment with SCF. All patients had severe perennial nasal symptom and had abundant mast cells (grading 3+) in nasal swab [27]. They were positive only to house dust or mite class 3 or more reactivity, as confirmed by immunoassay for specific IgE antibodies to JCP, house dust, mite, orchard grass, ragweed, mugwort and Alternaria. Patients had no immunotherapy and stopped the medicine at least 1 month before this study. All patients had the operation of extensive resection of inferior turbinates for improvement of blocked nose.

Informed consent was obtained from all patients and the research protocol was approved by the ethics committees of Nippon Medical School.

Methods

Nasal scrapings and preparation of nasal submucosa

Epithelial specimens were obtained by gently scraping of the nasal inferior turbinate with a small curette (Nagashgima Co., Tokyo, Japan) until the specimen filled the cup of the curette. With this procedure, about 6 mg (wet weight) of the epithelial layer was obtained (histamine contain >70 ng/specimen). Nasal scraping was taken before the operation of extensive resection of inferior turbinate's.

For tissue samples of the nasal subglandular region, the nasal mucosa together with the periosteum of the swollen inferior turbinate was removed from the turbinate bone during surgery to reconstruct the nasal blocked nose. The deeper areas underlying the grandular layer of the nasal mucosa (nasal submucosa) were separated using scissors, and cut into small specimens (2 mm to 3 mm diameter, total histamine contain of two small pieces >200 ng) immediately after the operation.

Solutions used for histamine and leukotriene release study

CaMgfree HEPES solution; HEPES (10 mM N-2hydroxyethyl piperrazine-N'-2-ethanesulfonic acid) -buffered Tyrode's with 0.1% Bovine Serum Albumin (BSA) without Ca2+ and Mg2+ (composed of 137 mmol/L NaCl, 5.6 mmol/L glucose, 2.7 mmol/L KCl, 0.4 mmol/l HaHPO4, pH 7.4).

Ca^{2+} Mg^{2+} HEPES solution; HEPES(10 mM N-2hydroxyethyl piperrazine-N'-2-ethanesulfonic acid)-buffered Tyrode's with 0.1% Bovine Serum Albumin (BSA) with Ca^{2+} (CaCl2 1.8 mM) and Mg^{2+} ($MgCl_2$ 0.5 mM) (composed of 137 mmol/L NaCl, 5.6 mmol/L glucose, 2.7 mmol/L KCl, 0.4 mmol/l $HaHPO_4$, pH 7.4).

Histamine release from nasal scrapings and nasal submucosa

Histamine release studies from nasal scrapings or nasal submucosa were duplicately done as previous described [6,12]. Nasal scrapings placed on piece of filter paper (4 mm × 4 mm, No4; Tokyo Rosh Co. Tokyo, Japan) (histamine contain >70 ng/specimen) or two small pieces of nasal submucosa (histamine contain >200 ng) were placed into a small plastic tube contained 900 µl of pre-warmed CaMgfree HEPES solution: for 20 min at 37°C to remove the histamine outside mast cell in nasal scrapings or nasal submucosa. Then nasal scrapings or nasal submucosa were placed into 300 µl of pre-warmed Ca^{2+} Mg^{2+} HEPES solution: Together 2.2 ng to 2.2 µg Protein Nitrogen (PN)/ml (final concentration) of Dp extract (gift from Torii Pharmaceutical Co., Tokyo, Japan), or without mite extract as control for 30 min at 37°C. The reaction was terminated with 600 µl of cold CaMgfree HEPES solution. The tube was centrifuged at 200 g for 3 min and the supernatant was removed and used histamine assay (A). Remaining nasal scraping or nasal submucosas at the bottom were suspended in 900 µl of cold CaMgfree HEPES solution, then boiled and used residual histamine assay (B).

The histamine content of A and B were determined by the method of May et al. with modification [28]. Histamine extracted was taken up in high performance liquid chromatography mobile phase (40% methanol in water containing 0.042 mol/l acetate buffer pH 4.0) and chromatography was performed on a YMC Packed Column A-302 (Yamamura Chemical Laboratories, Kyoto, Japan). The flow rate was an ml/min and the fluorescence was monitored at 460 nm with excitation at 360 nm.

Histamine release was expressed as a percentage of the total content in a nasal scraping or nasal deep mucosa and calculated as: Histamine in A ÷ (Histamine in A + Residual histamine in B) × 100.

Leukotriene release from nasal scrapings and nasal submucosa

Leukotriene release studies from nasal scrapings or nasal submucosa were duplicatedly done as previous described [6,12]. Nasal scrapings on piece of filter paper or two small pieces of nasal submucosa were placed into a small plastic tube contained 900 µl of pre-warmed CaMgfree HEPES solution for 20 min at 37°C to remove the histamine and leukotriene outside mast cell in nasal scrapings or nasal submucosa. Then nasal scrapings or nasal submucosa were placed into 450 µl of pre-warmed Ca^{2+} Mg^{2+} HEPES solution: Together with 2.2 ng to 2.2 µg(PN)/ml of Dp extract (final concentration), or without mite extract as control for 30 min at 37°C. The reaction was terminated with 900 µl of cold CaMgfree HEPES solution. The tube

was centrifuged at 200 g for 3 min and 450 µl of the supernatant was separated for leukotriene assay and frozen at -50°C, and residual 900 µl of the solution for the histamine assay. Remaining nasal scraping or nasal submucosas at the bottom were suspended in 900 µl of cold CaMgfree HEPES solution, then boiled and used residual histamine assay.

Leukotriene was measured by radioimmunoassay kit (DuPont NEN Research)

To relate LT release from nasal scraping or nasal submucosa to mast cell number in both tissues, we expressed LT content (C) released from nasal scraping or nasal submucosa in relation to total histamine content (D) in the tissue and calculated as: C÷D (pg/ng).

Effect of pretreatment with SCF on histamine and leukotriene release from nasal submucosa

To make sure in detail the effect of SCF on histamine and leukotriene release in nasal submucosa, an experiment was performed as described below.

Two small pieces of nasal submucosa in each of five small plastic tubes were placed into contained 900 µl of pre-warmed CaMgfree HEPES solution for 20 min at 37°C to remove the histamine outside mast cell in nasal submucosa. Then nasal submucosa were placed into 450 µl of pre-warmed Ca^{2+} Mg^{2+} HEPES solution in first, 405 µl in second and third, 382.5 µl in fourth and 360 µl in fifth. Then 45 µl of 1000 ng/ml of SCF (100 ng/ml of final concentration) in third, and 22.5 µl of 1000 ng/ml of SCF in fourth, and 45 µl of 1000 ng/ml in fifth tube at 37°C for 15 min. Then 45 µl of Dp extract 2.2 µg PN/ml in second, fourth and fifth tube (final concetration; 0.22 µgPN/ml) was added and incubated for 30 min at 37°C. Four-hundred fifty microliter was uniformly placed in each of the five tubes.

The reaction was terminated with 900 µl of cold CaMgfree HEPES solution. The tube was centrifuged at 200 g for 3 min and 450 µl of the supernatant was separated for LT assay and frozen at -50°C, and residual 900 µl of the solution for the histamine assay. Remaining nasal submucosa at the bottom were suspended in 900 µl of cold CaMgfree HEPES solution, boiled and used remaining histamine assay.

Histamine release and LT release were expressed as above mentioned.

Statistical analysis

For comparison of percent histamine release and LT release from nasal scrapings and nasal submucosa among concentrations of mite antigen extract, and from nasal submucosa among mite allergen alone, SCF and SCF plus mite allergen, t-test were used.

Results

Net Percentage Histamine Release (NPHR) from nasal scrapings and preparation of nasal submucosa

With nasal scrapings taken from 8 allergic patients, Dp extract at 2.2 ng PN/ml to 2.2 µg PN/ml caused a concentration related increased in NPHR. Averages and standard errors of NPHR from nasal scrapings were 0.58 ± 0.6% at 2.2 ngPN/ml, 5.7 ± 1.5 % at 22 ngPN/ml, 16.9 ± 2.5% at 0.22 µgPN/ml and 18.1 ± 1.8% at 2.2 µgPN/ml of Dp extract. NPHR was higher at concentration of 22 ngPN/ml than 2.2 ngPN/ml (p=0.003, t-test) and 0.22 µgPN/ml than 22 ngPN/ml (p=0.0016) of Dp extract. However, there was no statistical difference in NPHR between the concentrations of 0.22 µgPN/ml than 2.2 µgPN/ml. On the other hand, averages and standard errors of NPHR from nasal submucosa in same patients were -1.0 ± 1.4% at 2.2 ngPN/ml, -0.6 ± 0.7% at 22 ngPN/ml, 0.5 ± 1.2% at 0.22 µgPN/mgPN/ml, 2.4 ± 1.3% at 2.2 µgPN/ml of Dp extract. There was no statistical difference in NPHR among at concentrations of 2.2 ngPN/ml to 2.2 µgPN/ml (Figure 1).

Net Leukotriene Release Per Total Histamine (NLtRPH) from nasal scrapings and nasal submucosa

With nasal scrapings taken from 8 allergic patients, Dp extract at 2.2 ngPN/ml to 2.2 µgPN/ml caused a concentration related increased in NLtRPH. Averages and standard errors of NLtRPH from nasal scrapings were 0.29 ± 0.2 pg/ng at 2.2 ngPN/ml, 2.31 ± 0.57 pg/ng at 22 ngPN/ml, 11.58 ± 3.73 pg/ng at 0.22 µgPN/ml and 11.66 ± 3.5 pg/ng at 2.2 µgPN/ml of Dp extract. NLtRPH was higher at concentration of 22 ngPN/ml than 2.2 ngPN/ml (p=0.0035) and 0.22 µgPN/ml than 22 ngPN/ml (p=0.014) of Dp extract. However, there was no statistical difference in NLtRPH between the concentration of 0.22 µgPN/ml than 2.2 µgPN/ml. Whereas averages and standard errors of NLtRPH from nasal submucosa in same patients were 0.02±0.05 pg/ng at 2.2 ngPN/ml, 0.05±0.1pg/ng at 22 ngPN/ml, 0.18±0.1 pg/ng at 0.22 µgPN/ml, 0.08±0.06 pg/ng at 2.2 µgPN/ml of Dp extract. There was no statistical difference in NLtRPH among concentrations of 2.2 ngPN/ml to 2.2 µgPN/ml (Figure 2).

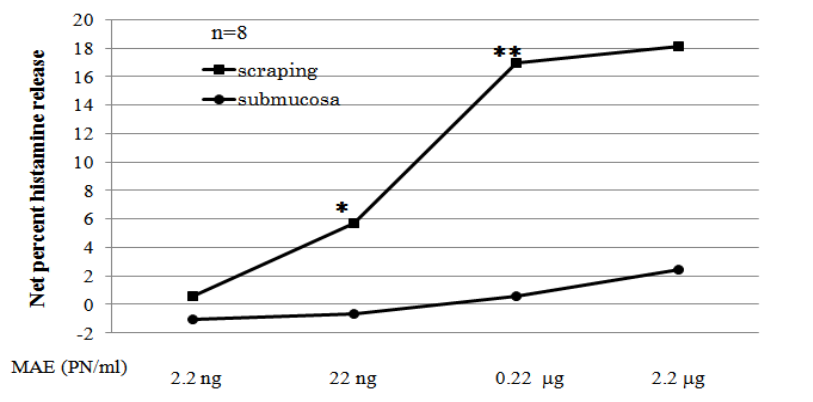

Figure 1: Net Percentage Histamine Release (NPHR) from nasal scrapings and preparation of nasal submucosa NPHR from nasal scrapings was significantly higher at concentration of 22 ngPN/ml than 2.2 ngPN/ml (*p=0.003, t-test) and 0.22 µgPN/ml than 22 ngPN/ml (**p=0.0016) of Dp extra.
There was no statistical difference in NPHR from deep nasal mucosa among the concentrations of 2.2 ngPN/ml to 2.2 µgPN/ml of Dp extra.
Dp: *Dermatophagoides pteronyssinus*; PN: Protein Nitrogen

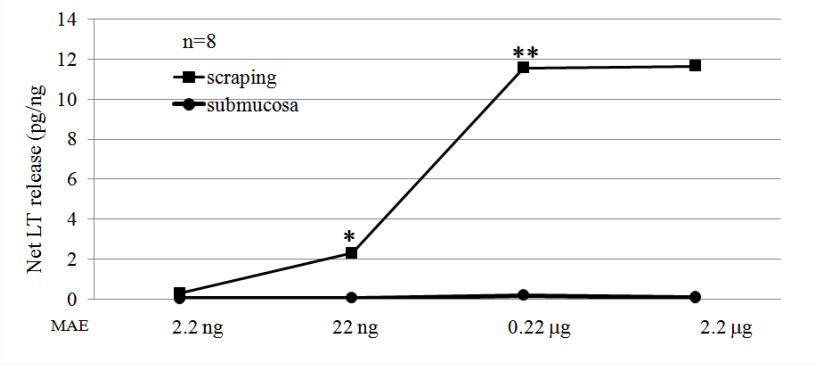

Figure 2: Net Leukotriene Release Per total Histamine (NLtRPH) from nasal scrapings and the nasal Submucosa NLtRPH was higher at concentration of 22 ngPN/ml than 2.2 ngPN/ml (*p=0.0035) and 0.22 µgPN/ml than 22 ngPN/ml (**p=0.014) of Dp extra from nasal scrapings.
There was no statistical difference among concentrations of 2.2 ngPN/ml to 2.2 µgPN/ml from nasal submucosa.

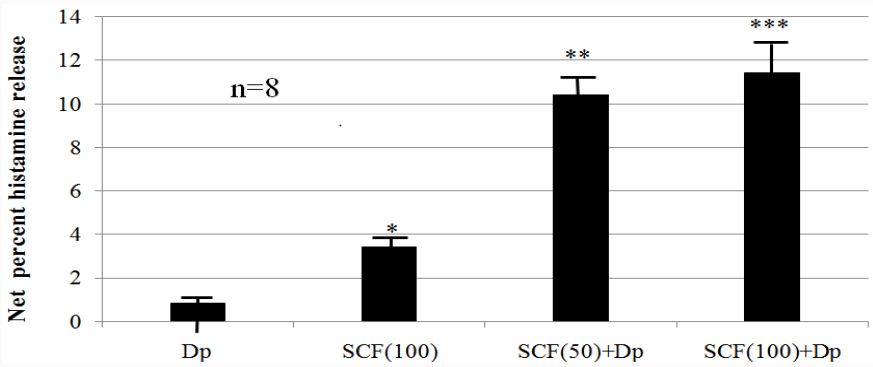

Figure 3: Effect of pretreatment with SCF on net histamine released from nasal submucosa to Dp extract (final concentration 0.22 µg PN/ml).
*NPHR for SCF (100 ng/ml) alone was significantly higher than Dp alone (p=0.035)
**NPHR for SCF (50 ng/ml) plus Dp was significantly higher than Dp alone (p=0.00078) and SCF (100 ng/ml) alone (p=0.0001).
***NPHR for SCF (100 ng/ml) plus Dp was significantly higher than allergen alone (p=0.00034) and SCF (100 ng/ml) alone (p=0.0000087).

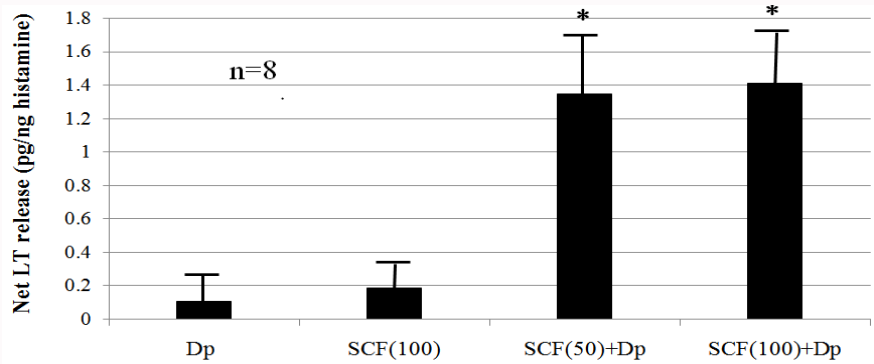

Figure 4: Effect of pretreatment with SCF on net leukotriene released from nasal submucosa to Dp extra (final concentration 0.22 µgPN/ml)
*NLtRPH for SCF (50 or 100 ng/ml) plus Dp was significantly higher than Dp alone (p<0.01) and SCF (100 ng/ml) alone (p<0.01).

Effect of pretreatment with SCF on histamine and leukotriene release from nasal submucosa

Effect of pretreatment with SCF on NPHR and NLtRPH from nasal submucosa was studied.

Given the less-responsiveness of the nasal submucosa to allergen challenge, we tested if pre-treatment with SCF for 20 min would alter responsiveness. NPHR and stand error from nasal deep mucosa of 8 patients was 0.8 ± 0.9% for Dp extract (0.22 µg PN/ml) alone, 3.4 ± 1.0% for SCF (100 ng/ml) alone, 10.4 ± 1.4% for SCF (50 ng/ml) plus Dp extract, 11.4 ± 1.5% for SCF (100 ng/ml) plus Dp extract. NPHR with SCF (50 ng/ml and 100 ng/ml) plus Dp extract was significantly higher than Dp extract alone (p=0.00078, p=0.00034) and SCF (100 ng/ml) alone (p=0.0001, p=0.0000087) (Figure 3).

Also, NPHR with SCF (100 ng/ml) alone was higher than Dp extract alone.

There was no significantly different between SCF (50 ng/ml) plus Dp extract and SCF (100 ng/ml) plus Dp extract.

NLtRPH and stand error from nasal submucosa of same 8 patients was 0.10 ± 0.18 pg/ng for Dp extract (0.22 µg PN/ml) alone, 0.18 ± 0.18 pg/ng for SCF (100 ng/ml) alone, 1.34 ± 0.39 pg/ng for SCF (50 ng/ml) plus Dp extract, 1.41 ± 0.35 pg/ng for SCF (100 ng/ml) plus

Dp extract. NLtRPH for SCF (50 or 100 ng/ml) plus Dp extract was significantly higher than only Dp extract ($p<0.01$) and only SCF (100 ng/ml) ($p<0.01$) (Figure 4).

However, NLtRPH with SCF (100 ng/ml) alone was not significantly higher than Dp extract alone.

There was no significantly different between SCF (50 ng/ml) plus Dp extract and SCF (100 ng/ml) plus Dp extract.

Discussion

In the past Okuda advocated the so-called nasal surface theory. He reported the spray with histamine solution on the nasal surface induced sneezing and nasal discharge but not induced the injection with same solution into nasal mucosa, and the antigen was not observed in the nasal submucosa when the antigen provocation was positive, whereas observed in the nasal submucosa of allergic patients when the reaction with the antigen was negative or no allergic subjects. He thought that the trigger of nasal manifestations must be localized at the nasal surface by the increased of basophils and mast cells there [7,16,17,18].

In 1980s, metachromatic cells in the epithelium, which was stained by Dye-binding after fixation by 99% methyl alcohol, Mota's lead acetate or Carnoy's fixation but not stained 66% formalin fixation, increased in seasonal or perennial nasal allergic patients [1,2].

We reported that with respect of formalin sensitivity of subsequent dye binding, in human nasal epithelium there are over than 80% Formalin-Sensitive Mast Cells (FSMC) and 10% Formalin Resistant Mast Cell (FRMC) [1]. We also reported that means of the proportion of MCT, MCTC, basophil in dispersed cells from nasal scrapings of allergic rhinitis were 83%, 10%, and 7% respectively [6]. Whereas in the nasal submucosa over than 90% of all metachromatic cells are FRMC or MCTC [1,5].

Bentrew et al. [11] reported the number of MCT in the nasal epithelium increased in seasonal allergic rhinitis but not MCT and MCTC in nasal submucosa compared with control subjects [11]. Moreover, treatment of steroid for allergic rhinitis and allergic asthma significantly reduced the number of FSMC or MCT in respiratory epithelium but not FRMC or MCTC in respiratory submucosa despite the improvement in symptoms [13-15]. Steroid treatment did not reduce chemical mediators release from human lung fragments and purified human lung mast cells [29,30]. The antigen was not observed in the nasal submucosa when the antigen provocation was positive, whereas observed in the nasal submucosa of allergic patients when the reaction with the antigen was negative [16]. By electron microscopic observation, Kawabori et al. mentioned mast cell in allergic nasal epithelium and in the subepithelial layer were degranulated after antigen provocation, whereas few cell with degranulation in nasal deep tissue [31]. These might indicate the MCTC in nasal submucosa of allergic rhinitis was no proliferative and no sensitive by allergic response.

We showed histamine and leukotriene release by using nasal scrapings with abundant MCT [6,12].

In this study, using nasal surface mast cells, mite allergen-induced histamine and LT release was in a dose dependent manner and optimal point of the dose of mite antigen for both mediators release was 0.22 μg PN/ml. On the other hand, allergen-induced histamine and LT release from nasal submucosa was insignificant, even at the highest dose allergen (2.2 μg PN/ml).

Mast cell in nasal surface was high responsive to specific antigen. In fact, mast cells in nasal submucosa was low responsive.

Mite antigen did not induce histamine and LT release from nasal submucosa. However, the pretreatment of SCF induced these mediators with mite antigen from nasal submucosa though the contents of both mediators were smaller compared with nasal scrapings. If low reactive mast cells in nasal submucosa was caused from the antigen had not fully penetrated to nasal submucosa, SCF pre-incubation did not enhance antigen-mediated histamine and LT release from mast cell in nasal submucosa.

Bischoff et al. made similar observations to ours with mast cell in nasal submucosa, namely that SCF pre-incubation enhanced IgE-mediated histamine and LT release from mast cells of human lung and intestine [21,23].

Previously, we and others reported that nasal epithelial cells express SCF mRNA and protein and that this correlated with mast cell numbers in the nasal scrapings [19,20]. In our report [20], immunohistochemical staining for SCF in nasal turbinate was seen stronger in the epithelium of nasal allergic patients than nasal submucosa.

This might mean that SCF produced from the epithelial cell as exogenous factor direct affected the proliferation and activation of MC in allergic inflammatory in the nose, whereas MC in the nasal submucosa might not be affected with exogenous SCF.

Acknowledgement

We thank Professor A. D. Befus, University of Alberta, Canada for review of the manuscript. Mrs. T. Murayama, Secretary of Musashikosugi Hospital ENT department assisted with data collection, and staffs in the clinic assisted with staining of nasal swabs.

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