

Tryptase in nasal lavage fluid after local allergen challenge

Relationship to histamine levels and TAME-esterase activity

S. JULIUSSON¹, K. HOLMBERG², C. R. BAUMGARTEN³, M. OLSSON⁴, I. ENANDER⁵
& U. PIPKORN⁶

¹ENT-Department, Sahlgrenska Hospital, Göteborg, ²ENT Department, Lundby Hospital, Göteborg, Sweden, ³Department of Clinical Immunology, Freie Universität, Berlin, Germany, ⁴Department of Clinical Immunology, University Hospital, Lund, Sweden, ⁵Pharmacia Diagnostics, Uppsala, Sweden and ⁶ENT-Department, University Hospital, Lund, Sweden

The activation of mast cells is generally considered to be an important trigger mechanism in the immediate allergic response. This study focused on the determination of three markers of mast cell activation after an allergen challenge. Nasal allergen challenges were performed in 25 subjects with seasonal allergic rhinitis using three allergen doses increasing in 10-fold steps in a standardised nasal lavage model for the subsequent recovery of the markers of mast cell activation. The levels of histamine and tryptase in the nasal lavage fluid were determined using radioimmunoassays, while the TAME-esterase activity was determined using a radiochemical technique. The nasal symptoms obtained on challenge were assessed using a scoring technique. The allergen challenge resulted in significant increases in the levels of all three markers, tryptase, histamine and TAME-esterase. In the individual measurements after the challenges there was a highly significant correlation between the TAME-esterase levels and the tryptase levels ($r = 0.71$; $P < 0.001$), while the generation of histamine and tryptase was not significantly correlated. When comparing the cumulative generation of the three markers, significant correlations were found between all three. Allergen challenges in six non-allergic controls using the same technique did not result in any increase in tryptase levels. The findings suggest that the determination of tryptase in nasal lavage fluid may be a valuable indicator of mast cell activation in the upper airways.

Key words: allergic rhinitis; histamine; mast cell activation; mediators; TAME-esterase; tryptase.

Accepted for publication 24 December 1990

The activation of mast cells is generally considered to be an important trigger mechanism in the immediate allergic response (7, 8, 13). The activation of mast cells by the interbridging of surface IgE on the mast cells leads to the release of preformed mediators such as histamine (13). It has also been shown that the activation of mast cells is accompanied by the release of tryptase, the major neutral protease in the mast cells (21, 24). It has been suggested that this can be used as a marker of mast cell activation,

since most mediators including histamine may also come from other sources, such as basophils. Furthermore, in contrast to several of the other mediators, the half-life of released tryptase is considerably longer and may therefore be detected within another time-frame than other markers of cell activation (24).

The most accessible part of the airway mucosa is that in the upper airways, which makes it suitable for studies of the pathophysiology of allergic airways mucosa (16). It is pos-

sible to study pathophysiological events by repeated sampling of cells and cell surface liquids in relation to functional measurements both in the challenge setting and during natural allergen exposure (2, 15, 17, 18). It has also been suggested that it is possible to show changes in local tryptase levels as a result of the allergen exposure of nasal mucosa (5). The aim of the present study was to explore any dose-response relationship between allergen exposure and the local release of tryptase. Further, we intended to study the relationship between the local generation of tryptase and the levels of histamine and TAME-esterase activity.

MATERIAL AND METHODS

Study design

The study was designed as a nasal allergen challenge study of 25 patients with strictly seasonal allergic rhinitis. A similar procedure was used in six normal controls. A nasal lavage technique was used to monitor changes in local levels of tryptase, histamine and TAME-esterase activity. The study was performed during pollen-free winter months. At least 2 weeks after a preparatory interview, including a diagnostic allergen challenge, the patients were subjected to the nasal allergen challenge of this study.

Subjects

Twenty-five patients (19 men and 6 women), aged 21–47 years (mean age 27.8 years), with strictly seasonal allergic rhinitis entered the study, which was performed during the winter months of January to March 1989. They all had a positive history of birch and/or timothy pollen allergy without any dermal or bronchial manifestations. Their allergy had been verified by a positive skin prick test and a positive nasal allergen challenge. Six non-atopic controls were studied. They were volunteers from the laboratory staff, five men and one woman with a mean age of 31.8 years (range 24–40). The nasal lavage fluids from the controls were only

analysed with regard to tryptase. None of the controls had nasal symptoms and they were all negative to nasal challenges with a panel of pollen extracts, as well as being negative in skin prick tests and RAST tests. Informed consent was obtained from each individual and the trial was approved by the Ethics Committee of Gothenburg University.

Nasal challenge procedure

A challenge procedure involving nasal lavages for the harvesting of mediators was used. The technique has previously been described in detail (14). Briefly, the procedure started with 3 quick lavages with 10 ml of saline, 5 ml into each nasal cavity, with the head tilted backwards during closure of the soft palate. Lavages were then performed at constant 10 min intervals. Each of the lavages, starting with the fourth, was immediately followed by the administration of a challenge agent, the sequence being oxymethazoline, diluent, diluent 100 Biological Units (BU) of allergen (birch or timothy), 1,000 BU and, finally, 10,000 BU. Each lavage was designed to harvest the result of the preceding challenge, as shown in Fig. 1. The lavage fluid was immediately chilled and then aliquoted and frozen for the subsequent analysis of the levels of histamine, tryptase and TAME-esterase activity. Each patient and normal subject was given a diary card on which the nasal symptoms were to be recorded at the same time as the lavages. The symptoms of nasal blockage, secretion, nasal itching and sneezing were rated on a 4-point scale where 0 = no symptoms, 1 = mild, 2 = moderate and 3 = severe symptoms. A composite symptom score was calculated from the individual symptoms.

Biochemical analysis

The TAME-esterase activity was measured in the nasal secretions using a radiochemical method originally described by Imanari et al. (9) and adapted for the nasal challenge procedure (14). The detection of histamine using

monoclonal antibodies was performed using an Immunotech Histamine RIA assay (Immunotech, Marseille, France) (10, 12). In the present study, the determination of histamine was carried out in accordance with the manufacturer's instructions. In the RIA, 100 μ l of nasal lavage fluid was acylated after centrifugation by adding 1 mg of acylating reagent and 50 μ l of borate buffer, pH 8.2. 1 ml of ^{125} I-labelled acetylated histamine was then added. The mixture was incubated for 18 h at 4°C in tubes coated with anti-acylated histamine monoclonal antibodies and then the tubes were counted in a gamma counter. Tryptase in the lavage fluid was analysed with a modified Pharmacia Trypsin RIAC assay (Pharmacia Diagnostics AB, Uppsala, Sweden) using two monoclonal antibodies for tryptase (16, 17). In the RIAC, 50 μ l of nasal lavage fluid, 50 μ l of sample diluent and 50 μ l of ^{125} I-labelled anti-tryptase antibody were simultaneously added to plastic tubes coated with anti-tryptase antibody. After incubation overnight, the tubes were washed three times and then counted in a gamma counter. The lower limit of detection for the assay is less than 0.5 ng/ml (Enander I. et al., to be published).

Statistics

The statistical analysis was performed on a microcomputer using a statistical software pack-

age. An analysis of variance was used to see whether there were any statistically significant differences between the repeated observations. A further analysis was made using Student's *t*-test (two-tailed). Any putative correlations between the levels of the different markers were analysed using a regression analysis.

RESULTS

As expected, the allergen challenges induced a dose-dependent increase in nasal symptoms as shown in Fig. 2. Correspondingly, there was a dose-dependent increase in TAME-esterase activity as well as in the levels of tryptase in the lavage fluid; this is also shown in Fig. 2. Compared to the levels found after diluent challenge, there was a statistically significant increase in TAME-esterase after all three allergen challenges and in tryptase after the two highest dose levels. The levels of histamine in the lavage fluid did not show a dose-response relationship. A significant, 3-fold, increase ($P < 0.001$) was noted after the first allergen challenge but no further rise was noted, as shown in Fig. 2. The individual levels of tryptase in the lavage fluid after the challenges with diluent and the three allergen doses are shown in Fig. 3. Of specific interest is the observation that there was no detectable increase in tryptase level in the first lavage fluid. Although symptoms were present after the highest allergen dose in all the allergic individuals who were tested, the tryptase levels of four of the subjects were not above the limit of detection in the corresponding lavage fluid. The results from the controls are also shown in Fig. 3. As expected, none of the controls exhibited any increase in tryptase level in the lavage fluid.

The relationships between the individual levels of tryptase, TAME-esterase activity and histamine obtained after challenge with diluent and the three allergen doses are given in Fig. 4. As shown, there was a fairly strong correlation between TAME-esterase and tryptase levels ($r = 0.71$; $P < 0.0001$; $n = 100$), while the relationship between the levels of tryptase and histamine was much weaker ($r = 0.30$; $P < 0.001$; $n = 100$). The corresponding values for TAME-

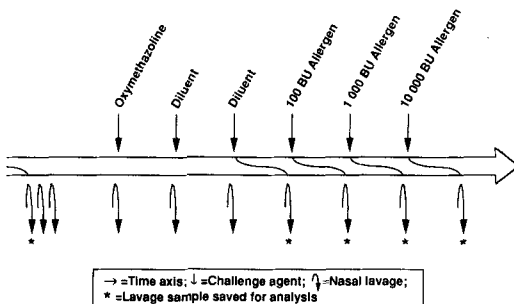


Fig. 1. The nasal lavage procedure. After 3 quick lavages with 5 ml of saline into each nasal cavity, the lavages were performed at constant 10 min intervals. Each lavage, starting with the fourth, was immediately followed by the administration of a challenge agent.

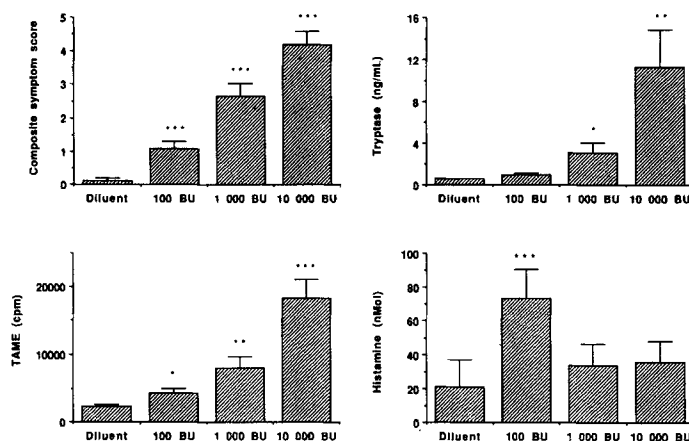


Fig. 2. Combined figure showing the symptoms and various markers of mast-cell activation after provocation with diluent and increasing doses of allergen. The upper panels show the resulting symptoms and the levels of trypase obtained. The lower panels show the TAME-esterase and histamine levels. As shown, increasing levels of allergen, delivered topically into the nasal cavity, resulted in increasing nasal symptoms and increasing levels of the various markers in the nasal lavage fluid. * $P < 0.05$, ** 0.01 , *** 0.001 (diluent challenge levels versus allergen challenge levels).

esterase levels and histamine did not demonstrate any statistically significant relationship ($r = 0.15$; $P > 0.05$; $n = 100$)

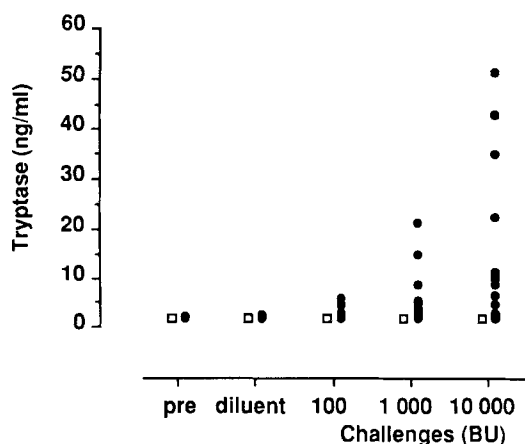


Fig. 3. Individual values of trypase levels in nasal lavage fluid after local challenge with increasing levels of allergen in 25 patients (●) with strictly seasonal allergic rhinitis and 6 normal subjects (□). As shown, increasing doses of allergen resulted in increasing numbers of patients with levels above the limit of detection of the assay. None of the controls had increased levels of trypase in the lavage fluid as a result of the allergen exposure.

Since there might be a difference in timing in the generation of the different mediators, a corresponding analysis of relationships was also made between the total amount of the markers generated after the three allergen challenges in the 25 patients. These figures are presented in Table 1. A statistically significant correlation was then present between the two mast cell-derived markers trypase and histamine. Since trypase was measurable in the largest number of patients after challenge with the highest allergen dose, these levels were correlated to the composite symptom score for this allergen challenge ($r = 0.12$; $P > 0.05$; $n = 25$) as well as to a cumulative composite score ($r = 0.26$; $P > 0.05$; $n = 25$). No significant correlation was obtained.

DISCUSSION

In this study, we were able to demonstrate the release of trypase in the human nose after nasal allergen challenge in allergic individuals but not in healthy controls. Further, we were able to demonstrate significant relationships between the generation of trypase, TAME-esterase activity and histamine.

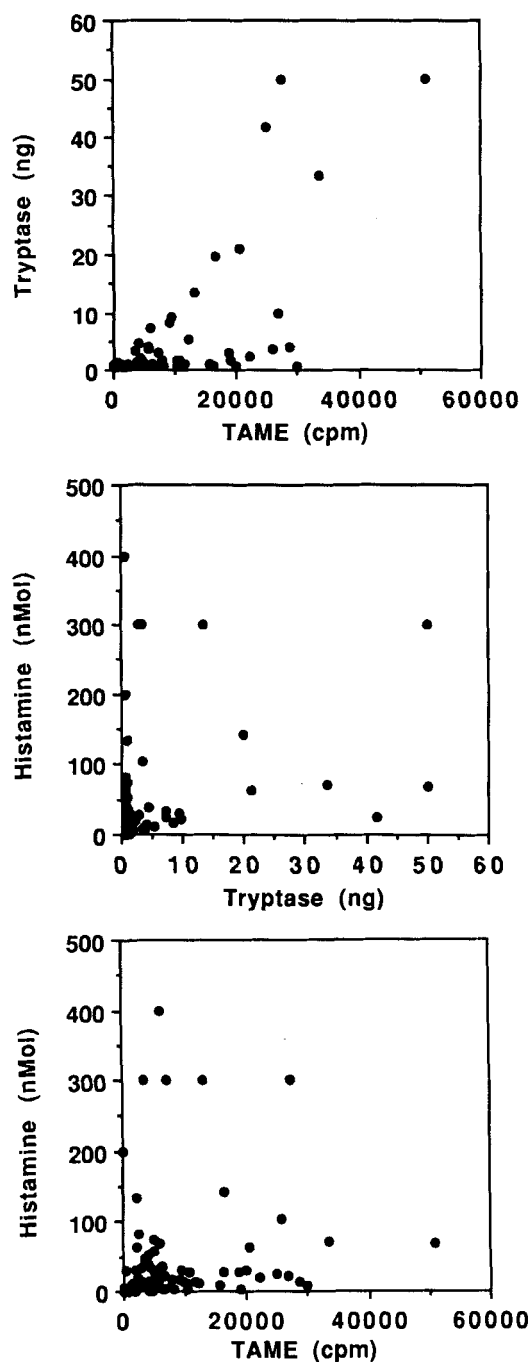


Fig. 4. Combined figure showing the relationships between the individual levels of histamine, tryptase and allergen in nasal lavages after challenge with diluent and increasing doses of allergen. The correlation coefficients between TAME esterase and tryptase were $r = 0.71$ ($P < 0.0001$; $n = 100$), between tryptase and histamine $r = 0.30$ ($P < 0.001$; $n = 100$), and between the TAME-esterase levels and histamine $r = 0.15$ ($P > 0.05$; $n = 100$).

It has previously been shown that it is possible to measure increased levels of tryptase in nasal lavage fluid after local allergen challenge (5). In the present study, we increased the number of subjects studied, measured the levels after exposure to several allergen dose levels and also compared the results with other markers of the allergic inflammatory response, TAME-esterase activity and histamine. Furthermore, we sought specific relationships between the two mast-cell-derived markers, histamine and tryptase in relation to symptoms. Histamine was significantly increased in the lavage fluid after challenge with the lowest allergen dose, while a statistically significant rise in tryptase was only detectable after the second allergen challenge. This is in harmony with the findings of Castells et al. (5). The dissociation between histamine and tryptase levels may be due to technical factors, such as assay sensitivity. The difference in timing in the release process might well explain the lack of a strong relationship between the individual levels of histamine and tryptase (23). Another possible explanation for a difference in appearance is that the two markers may behave differently in terms of their ability to diffuse through the tissue to the locality where they are harvested (1). Nevertheless, the stronger relationship between the total generation of tryptase and histamine might indicate that these two markers are released together. These findings lend further support to the concept that mast cell activation is an essential part of the immediate allergic response. In this study we found a strong correlation between the individual levels of TAME-esterase and tryptase. This was not surprising since 25–30% of the TAME-esterase activity measured in the challenge situation represents

Table 1.

Correlation table for the total generation of the three markers, tryptase, histamine and TAME esterase after allergen challenge

Marker	Tryptase	Histamine	TAME
Tryptase	1		
Histamine	0.70**	1	
TAME	0.73**	0.36*	1

* $P < 0.05$, **0.01

tryptase activity, while the remainder largely represents plasma kallikrein activity (3).

Like previous reports on histamine levels in nasal lavage fluid (6, 11, 14), we found only a weak relationship between the severity of symptoms after the allergen challenges and the levels of tryptase. These findings appear to be at variance with the previous report by Castells et al. (5), who pointed to the possibility of a correlation between the severity of symptoms and the levels of tryptase. This was, however, based on results from only seven allergic subjects, using varying amounts of allergen for challenge in the individual patients. The lack of a strong relationship between the amount of mast cell-derived marker and nasal symptoms is not so surprising since there are several modulating steps between the initial trigger and the final symptomatology. It was also interesting to note that baseline tryptase (the first lavage, see Fig. 3) was not above the limit of detection. This appears to contrast with the findings relating to histamine. High and variable baseline histamine levels in nasal lavage fluid have confused several authors (4, 6, 11). However, this is a consistent finding (4, 11, 14, 19, 20) and the present finding of low tryptase levels in these lavages does not shed any light on the specific source of this histamine and its putative physiological function.

In this study, we have used an RIA with a monoclonal antibody to detect histamine in the nasal lavage fluid. This assay is specific to histamine and does not determine the first degradation product of histamine, methyl-histamine, in contrast to the traditional OPT reaction-based methods (26). This might well explain why we failed to obtain a clear-cut dose-response relationship after the allergen challenges. We were nevertheless able to demonstrate an increase in the histamine levels after allergen challenge.

In conclusion, the present study provides further evidence of mast cell activation in the initial nasal response to allergen exposure. We have shown a relationship between histamine and tryptase levels in nasal lavage fluid after allergen challenge, which might indicate a si-

multaneous release. Whether the determination of tryptase levels in nasal lavage fluid can also be used as a marker of mast cell activation during natural disease remains to be determined.

ACKNOWLEDGEMENTS

This study was supported by grants from the Swedish Medical Research Council (project 8308), the Medical Faculty of Gothenburg University and Glaxo Läkemedel AB, Göteborg, Sweden.

REFERENCES

- 1 Alter SC, Schwartz LB. Tryptase: An indicator of mast cell-mediated allergic reactions. In: Spector SL (ed.): *Provocative challenge procedures: Background and methodology*, pp 167-183. Futura Publishing Company, Mount Kisco, NY. 1989.
- 2 Anderson M, Svensson C, Andersson P, Pipkorn U. Objective monitoring of the allergic inflammatory response of the nasal mucosa in hay fever patients during natural allergen exposure. *Am Rev Respir Dis* 139, 911-914, 1989.
- 3 Baumgarten CR, Nichols RC, Naclerio RM, Lichtenstein LM, Norman PS, Proud D. Plasma kallikrein during experimentally-induced allergic rhinitis: Role of kinin formation and contribution to TAME-esterase activity in nasal secretions. *J Immunol* 137, 977-982, 1986.
- 4 Bisgaard H, Robinson C, Romeling F, Mygind N, Church M, Holgate ST. Leukotriene C4 and histamine in the early allergic reaction in the nose. *Allergy* 43, 219-227, 1988.
- 5 Castells M, Schwartz LB. Tryptase levels in nasal lavage fluid as an indicator of the immediate allergic response. *J Allergy Clin Immunol* 82, 348-355, 1988.
- 6 Davies RJ, Devalia JL. Histamine in nasal secretions; effect of methacholine and allergen. In: Mygind N, Pipkorn U (eds.): *Allergic and vasomotor rhinitis. Pathophysiological aspects*, pp 178-189. Munksgaard, Copenhagen 1987.
- 7 Enerbäck L, Pipkorn U, Granerus G. Intraepithelial migration of nasal mucosal mast cells in hay fever. *Int Arch Allergy Appl Immunol* 80, 44-51, 1986.
- 8 Enerbäck L, Pipkorn U, Olofsson A. Intraepithelial migration of nasal mucosal mast cells in hay fever. Ultrastructural observations. *Int Arch Allergy Appl Immunol* 81, 289-297, 1986.
- 9 Imanari T, Kaizu T, Yoshida H, Yates K, Pierce JV, Pisano JJ. Radiochemical assays for human urinary, salivary, and plasma kallikrein. In: Pisano JJ, Austen KF (eds.): *Chemistry and biology of the kallikrein system in health and disease*, pp 205-513. Washington, DC, Government Printing Office DHEW, publication no. (NIH) 76-791. 1976.
- 10 Lebel B, Bouschet J, Morel A, Chanal I, Godard I, Michel FB. Correlation between symptoms and the thresh-

- hold for release of mediators in nasal secretions during nasal challenge with grass-pollen grains. *J Allergy Clin Immunol* 82, 869-877, 1988.
- 11 Linder A, Strandberg K, Deuschl H. Variations in histamine concentrations in nasal secretion in patients with allergic rhinitis. *Allergy* 43, 119-126, 1988.
 - 12 McBride PT, Kaliner MA. Histamine determination: Immunologic methods. *ACI News* 2, 50-54, 1989.
 - 13 Mygind N. The allergic inflammatory reaction. Early and late reactions. In: Mygind N. *Essential allergy*, pp 48-50. Blackwell Scientific Publications, Oxford, 1986.
 - 14 Naclerio RM, Meier HL, Kagey-Sobotka A, et al. Mediator release after nasal airway challenge with allergen. *Am Rev Respir Dis* 138, 597-602, 1983.
 - 15 Naclerio RM, Togias A, Proud D, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 313, 65-70, 1985.
 - 16 Pipkorn U, Bisgaard H, Wihl JÅ. Nasal provocation testing and lavage technique. In: Mygind N, Pipkorn U (eds.): *Pathophysiological aspects of allergic and vasomotor rhinitis*, pp 149-164. Munksgaard, Copenhagen, 1987.
 - 17 Pipkorn U, Karlsson G, Enerbäck L. Nasal mucosal response to repeated challenges with pollen allergen. *Am Rev Respir Dis* 140, 729-736, 1989.
 - 18 Pipkorn U, Karlsson G, Enerbäck L. Cellular response of the human allergic mucosa to natural allergen exposure. *J Allergy Clin Immunol* 82, 1046-1054, 1988.
 - 19 Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N Engl J Med* 316, 1506-1510, 1987.
 - 20 Pipkorn U, Proud D, Schleimer RP, et al. Effects of short term systemic glucocorticoid treatment on human nasal mediator release after antigen challenge. *J Clin Invest* 80, 957-961, 1987.
 - 21 Schwartz LB, Atkins PC, Bradford R, Schalit M, Zweiman B. Release of tryptase together with histamine during the immediate cutaneous response to allergen. *J Allergy Clin Immunol* 80, 850-855, 1987.
 - 22 Schwartz LB, Bradford TR, Lee DC. Immunologic and physiochemical evidence for conformational changes occurring on conversion of human mast cell tryptase from active tetramer to inactive monomer: Production of monoclonal antibodies recognizing active tryptase. *J Immunol* (in press) 1990.
 - 23 Schwartz LB, Lewis RA, Seldin D, Austen KF. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. *J Immunol* 126, 1290-1295, 1981.
 - 24 Schwartz LB, Metcalf DD, Miller J, Earl H, Sullivan T. Tryptase levels as an indicator of mast cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 316, 1622-1626, 1987.
 - 25 Wenzel S, Irani AM, Sanders JM, Bradford TR, Schwartz LB. Immunoassay of tryptase from human mast cells. *J Immunol Methods* 86, 139-142, 1986.
 - 26 Allenmark S, Bergström S, Enerbäck L. A selective post-column o-phthalaldehyde derivatization system for the determination of histamine in biological material by high-performance liquid chromatography. *Anal Biochem* 144, 89-103, 1985.

Address:

Sigurdur Juliusson, M.D.
ENT-Department
Sahlgrenska Hospital
S-413 45 Göteborg
Sweden