

# Eosinophil Inflammation of Nasal Polyp Tissue: Relationships with Matrix Metalloproteinases, Tissue Inhibitor of Metalloproteinase-1, and Transforming Growth Factor- $\beta$ 1

Eosinophil and mast cell infiltrations are consistent findings in nasal polyp tissue. Previous studies have shown that matrix metalloproteinases (MMPs) may be involved in eosinophil infiltration in airway mucosa of asthmatic patients, and that transforming growth factor-beta1 (TGF- $\beta$ 1) induces extracellular matrix deposition in nasal polyp tissue. The aim of this study was to evaluate the role of MMPs and tissue-inhibitor of metalloproteinase-1 (TIMP-1) in association with TGF- $\beta$ 1, eosinophils and mast cell activation in nasal polyp tissue. Nasal polyp tissues from 20 patients who underwent polypectomies were collected and prepared into tissue homogenate. Eosinophil cationic protein (ECP) and tryptase levels were measured by CAP system (Pharmacia, Sweden). MMP-2, MMP-9, TIMP-1 and TGF- $\beta$ 1 levels were measured by enzyme-linked immunosorbent assay. MMP-2 was the predominant form of MMPs, followed by MMP-9 and TIMP-1. There were significant correlations between ECP, and MMP-9, MMP-2, TGF- $\beta$ 1 and tryptase, but not with TIMP-1. Significant correlations were noted between tryptase, and MMP-2, MMP-9, and TGF- $\beta$ 1, but not with TIMP-1. Close correlations were noted between TGF- $\beta$ 1, and MMP-9 and MMP-2, but not with TIMP-1. MMP-2, MMP-9, and TGF- $\beta$ 1 may contribute to eosinophil and mast cell migrations into nasal polyp tissue.

**Key Words :** Nasal Polyps; Matrix Metalloproteinases; Tissue-Inhibitor of Metalloproteinase-1; Transforming Growth Factor beta; Eosinophils

Young-Mok Lee\*, Sun-Sin Kim,  
Hyun-Ah Kim, Yu-Jin Suh, Soo-Keol Lee,  
Dong-Ho Nahm, Hae-Sim Park

Department of Respiratory Medicine and Allergy,  
Soonchunhyang University Hospital\*, Seoul;  
Department of Allergy and Clinical Immunology, Ajou  
University School of Medicine, Suwon, Korea

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## Address for correspondence

Hae-Sim Park, M.D.  
Department of Allergy and Rheumatology,  
Ajou University School of Medicine, San-5,  
Wonchondong, Paldalgu, Suwon 442-749, Korea  
Tel : +82.31-219-5196, Fax : +82.31-219-5154  
E-mail: hspark@madang.ajou.ac.kr

## INTRODUCTION

Nasal polyposis (NP) is a chronic inflammatory disease of the upper airway featuring inflammatory cell infiltration, tissue remodeling that includes extracellular matrix (ECM) accumulation and fibrosis (1). The inflammatory cellular infiltrate in nasal polyps has been shown to consist of eosinophils, lymphocytes, plasma cells, and mast cells to an extent which is similar to that observed in the bronchial mucosa of asthmatics (2-4), suggesting that inflammatory mechanisms of the two diseases may be similar (5). According to experimental models, it seems reasonable to speculate that the formation and growth of NPs require ECM accumulation (6).

The matrix metalloproteinases (MMPs) appear to be responsible for edema and cell transmigration, and ECM remodeling in asthmatic airways (7). Among the matrix metalloproteinases (MMPs), MMP-9, especially active MMP-9 expression, was clearly enhanced in nasal polyps compared with control mucosa (8). The activation of MMPs is inhibited by tissue inhibitors of metalloproteinases (TIMPs) that form a 1:1 complex with MMPs (9). Loss of coordination in the expression of MMPs and TIMPs is believed to generate tissue degra-

ation in inflammatory diseases. Eosinophil is one of the major sources of MMPs; MMP-9 was overexpressed by eosinophils accumulating in airway walls of asthmatics (10). Several *in vitro* studies have demonstrated that MMP-2 and MMP-9 were produced and activated by mast cells, and the possible involvement of mast cells in connective tissue degradation and fibrosis was suggested (11, 12).

Transforming growth factor- $\beta$  (TGF- $\beta$ ) is a chemoattractant for fibroblasts, stimulating fibroblast proliferation, and enhancing collagen laydown by fibroblasts. Of the TGF- $\beta$  isoforms, TGF- $\beta$ 1 synthesized by infiltrating eosinophils may contribute to stromal fibrosis and basement membrane thickening which are characteristic of nasal polyp (13). Another *in vitro* study showed that TGF- $\beta$ 1 induces MMP-9 proenzyme and MMP-2 (14). There have been few studies in relation to infiltrating cell activations and TGF- $\beta$ 1 on the specific effects of MMP-2, MMP-9, and TIMP-1 in nasal polyps, which shares certain similarities with asthma. The aim of the present study was to investigate the association between MMPs and TIMP-1, and inflammatory cell activation markers in nasal polyp tissue in relation to TGF- $\beta$ 1.

## MATERIALS AND METHODS

### Subjects

Nasal polyps were obtained from 20 patients with bronchial asthma, between the ages of 27 and 56 yr ( $40.0 \pm 3.4$  yr [mean  $\pm$  SEM]), who had complained of asthmatic symptoms during visits to the Allergy Clinic of Ajou University Hospital, Suwon, Korea (Table 1). Nasal polyps were identified by anterior rhinoscopy in all patients. Asthma was diagnosed according to the American Thoracic Society guidelines for the diagnosis of asthma (15). Skin prick test with common aeroallergens (Bencard, U.K.) was performed and atopy was defined as showing positive response ( $\geq 3+$  by Allergen/Histamine ratio) to two or more inhalant allergens. None of the study subjects had used topical steroids for at least 4 weeks prior to obtaining the polyp. The polyps, soon after surgical removal, were washed with normal saline to remove stagnant mucus. The middle portion of the polyp was cut off and immediately sent to a pathology laboratory to prepare the paraffin-embedded tissue. Half of them were made into tissue homogenate. All the subjects gave their informed consent as regulated by the Ajou University Hospital, Suwon, Korea.

### Preparation of nasal polyp tissue

The polyp tissue was frozen at  $-70^{\circ}\text{C}$  immediately after the operation. When prepared for the experiments, the samples were thawed, and ground in a homogenizer (POLYTRON, Switzerland) with phosphate-buffered saline (PBS, pH7.5) including 1% Triton X-100. They were centrifuged and the separated supernatant was kept at  $-70^{\circ}\text{C}$  to measure ECP, trypsinase, MMP-2, MMP-9, TIMP-1, and TGF- $\beta$ 1 levels.

### Enzyme-Linked Immunosorbent Assays for MMP-2, MMP-9, TIMP-1, and TGF- $\beta$ 1

Commercially available kits were used (MMP-2, MMP-9, and TIMP-1:RPN 2617, RPN 2614, and RPN 2611, respectively; Amersham Pharmacia Biotech, U.K., TGF- $\beta$ 1:Quantikine; R&D Systems, Minneapolis, U.S.A.). The MMP-2 and MMP-9 ELISA detects free proMMP-2, proMMP-9, and pro MMP-9-TIMP-1 complexes. It does not detect active MMP-2 or MMP-9. The TIMP-1 kit detects the total TIMP-1, free

TIMP-1, and MMP-9-TIMP-1 complexes. For all assays, optical density was measured with a spectrophotometer set at 450 nm. Quantification was performed by interpolation from a standard curve. The detection limit was 0.37 ng/mL for MMP-2, 0.6 ng/mL for MMP-9, 1.25 ng/mL for TIMP-1, and 7 pg/mL for TGF- $\beta$ 1.

Levels of protein were also measured by nephelometry (16) with a Beckman Array system (Beckman Instruments Inc., Brea, Calif). The data of MMPs, TIMP-1, and TGF- $\beta$ 1 concentrations in tissue homogenate of nasal polyp were presented as the ratio of protein measured, and were expressed as ng/mg protein concentration.

### ECP and tryptase assay

ECP and tryptase in tissue homogenate of nasal polyp were measured by fluoroimmunoassay using the Pharmacia CAP system (Pharmacia and Upjohn, Uppsala, Sweden). The lowest detection limits for ECP and tryptase measurements were 2 ng/mL and 4 ng/mL, respectively. The data of ECP and tryptase concentrations were presented as the ratio of protein measured, and were expressed as ng/mg protein concentration.

### Statistical analysis

The Mann-Whitney U test and ANOVA were applied using the SPSS version 7.0 (Chicago, U.S.A.) to evaluate the statistical differences among the data. The Pearson correlation analysis was applied to evaluate the statistical significance between the two values. A *p* value of 0.05 or less was regarded as significant.

## RESULTS

### Clinical features of the study subjects

The characteristics of the study subjects are shown in Table 1. No significant differences were noted in MMP-2, MMP-9, TIMP-1, TGF- $\beta$ 1 levels, and in molar ratio of MMP-9/TIMP-1 according to atopy status, duration of nasal symptom, and recurrence ( $p > 0.05$ , respectively).

### Correlations of MMP-2, MMP-9, and TIMP-1 levels, and inflammatory cell markers

The MMP-2 level ( $109.3 \pm 17.9$  ng/mg protein [mean  $\pm$  SEM]) was the highest in polyp tissue homogenate, followed by the MMP-9 ( $40.8 \pm 11.1$ ) and TIMP-1 ( $26.9 \pm 7.2$ ) levels ( $p < 0.05$ ). However, there was no significant difference between MMP-9 and TIMP-1 levels ( $p > 0.05$ ; Fig. 1A). The MMP-9 level in polyp tissue homogenate was significantly correlated with the TIMP-1 level ( $r = 0.44$ ,  $p < 0.05$ ; Fig. 1B). The ECP level in polyp tissue homogenate was significantly correlated

Table 1. Characteristics of study subjects

Number	20
Sex (male:female)	14:6
Age (yr)*	$40.0 \pm 3.4$
Atopy (presence/absence)	6/14
Duration of nasal symptom (yr)*	$2.8 \pm 0.4$
Recurrence (presence/absence)	8/12

\*Values are mean  $\pm$  S.E.

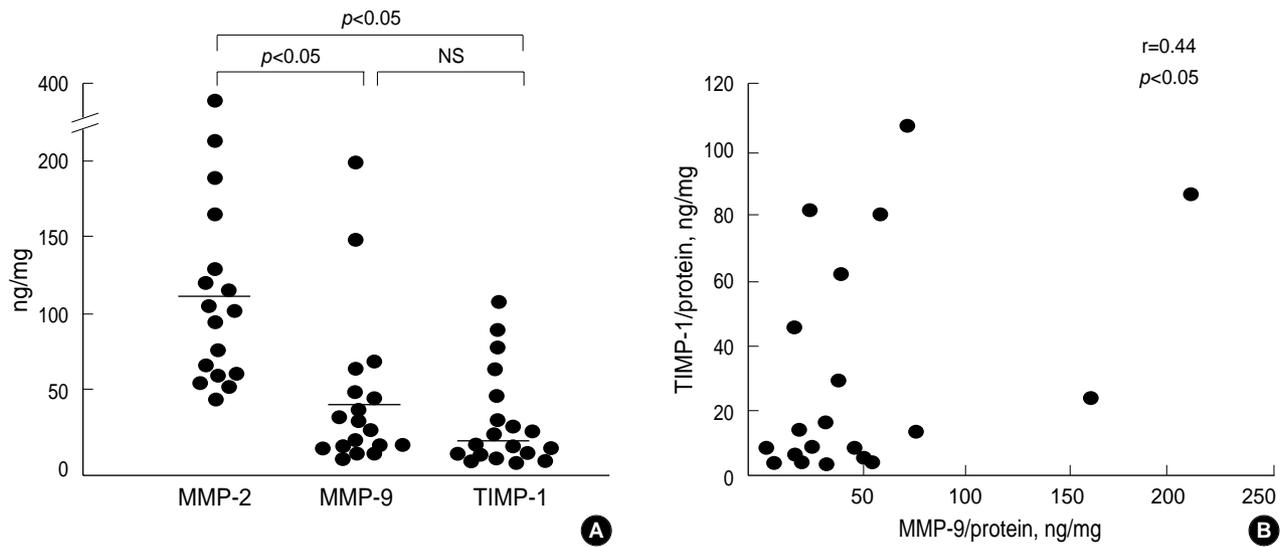


Fig. 1. Comparison of MMP-2, MMP-9, and TIMP-1 levels in nasal polyp tissue homogenate (A) and the correlation between MMP-9 and TIMP-1 level (B). Horizontal bars represent mean values.

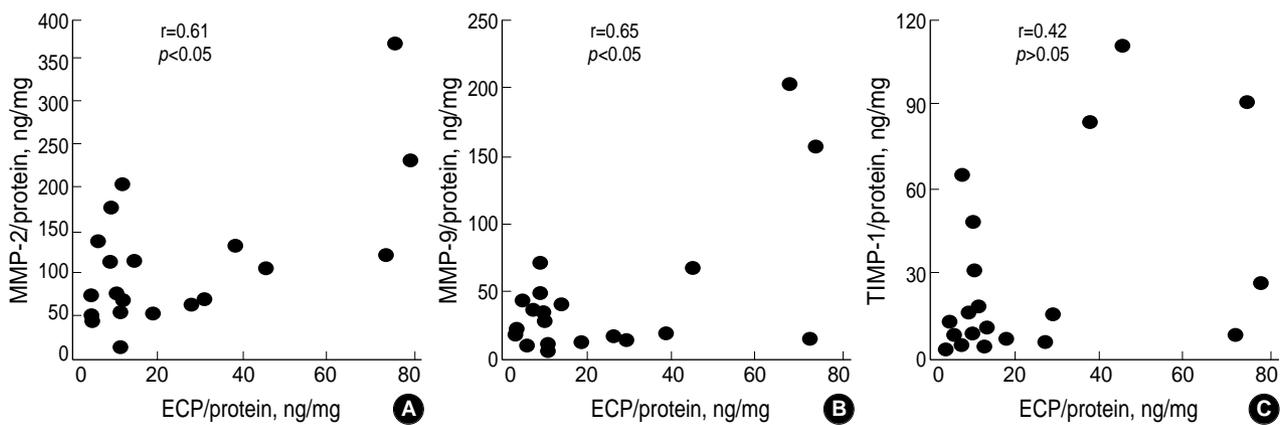


Fig. 2. Correlations between ECP, and MMP-2 (A), MMP-9 (B) and TIMP-1 (C) levels in nasal polyp tissue.

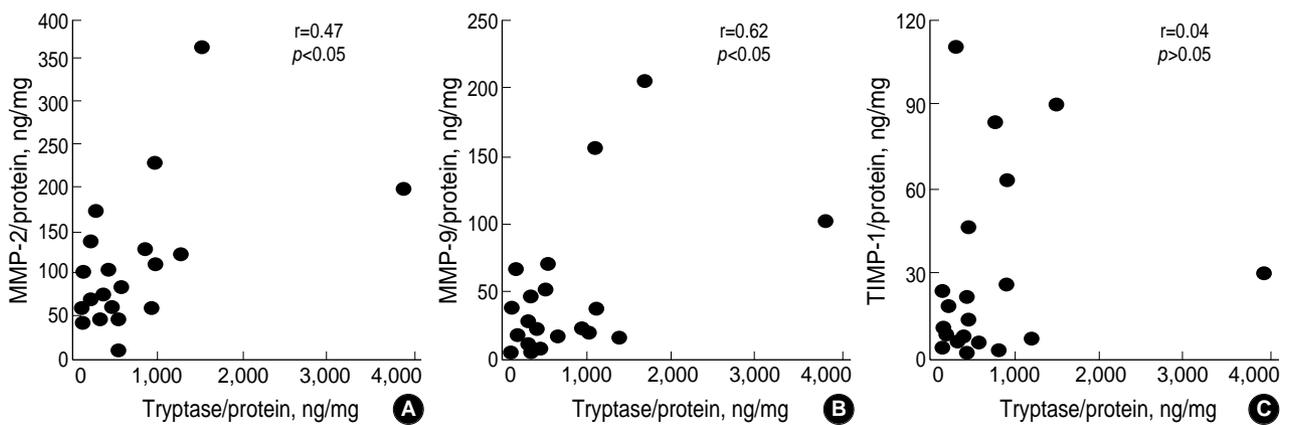


Fig. 3. Correlations between tryptase, and MMP-2 (A), MMP-9 (B) and TIMP-1 (C) levels in nasal polyp tissue.

with the MMP-2 ( $r = 0.61$ ,  $p < 0.05$ ) and MMP-9 ( $r = 0.65$ ,  $p < 0.05$ ) levels, but not with the TIMP-1 level ( $r = 0.42$ ,  $p > 0.05$ ) as shown in Fig. 2. Significant correlations were noted between

the tryptase level in polyp tissue homogenate, and MMP-2 ( $r = 0.47$ ,  $p < 0.05$ ), and MMP-9 ( $r = 0.62$ ,  $p < 0.05$ ), but not with TIMP-1 ( $r = 0.04$ ,  $p > 0.05$ ) level as shown in Fig. 3.

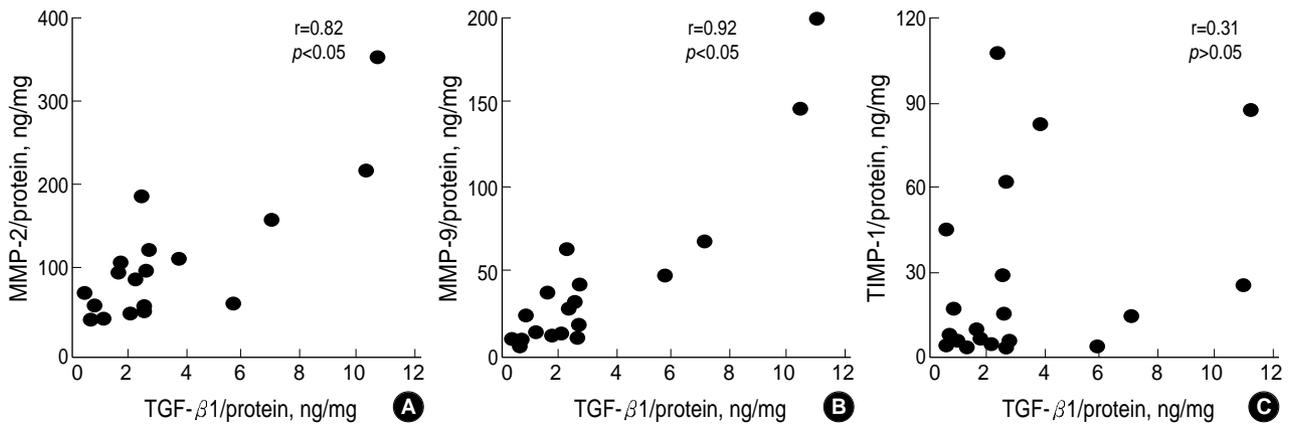


Fig. 4. Correlations between TGF- $\beta$ 1, and MMP-2 (A), MMP-9 (B) and TIMP-1 (C) level in nasal polyp tissue.

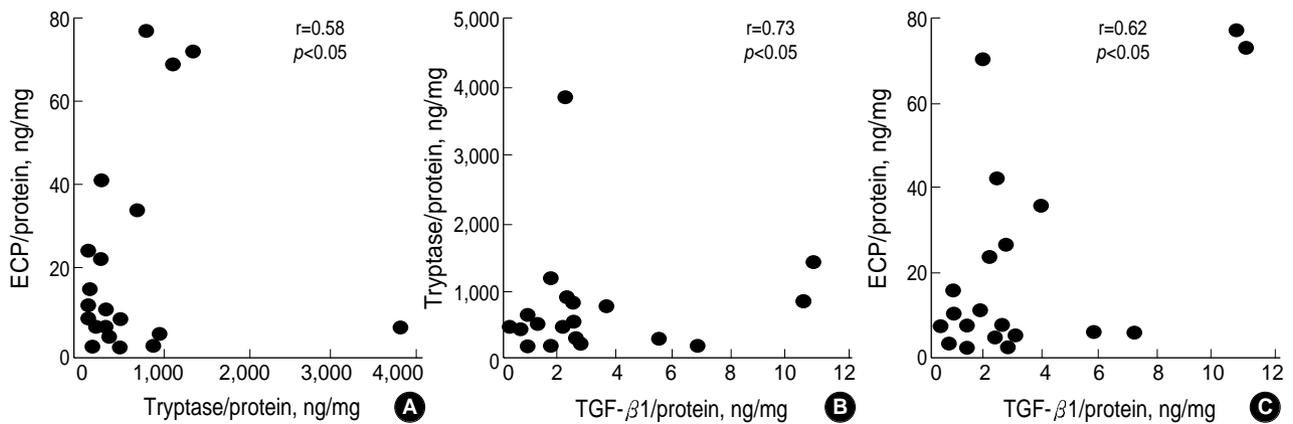


Fig. 5. Correlations between ECP and tryptase (A), TGF- $\beta$ 1 and tryptase (B), and ECP and TGF- $\beta$ 1 (C) levels in nasal polyp tissue.

### Correlations of TGF- $\beta$ 1, MMPs, and TIMP-1 levels, and inflammatory cell markers

As shown in Fig. 4, close correlations were also noted between the TGF- $\beta$ 1 level in polyp tissue homogenate, and the MMP-2 ( $r=0.82$ ,  $p<0.05$ ) and MMP-9 ( $r=0.92$ ,  $p<0.05$ ) levels, but not with the TIMP-1 level ( $r=0.31$ ,  $p>0.05$ ). Fig. 5 shows the correlations between the ECP, tryptase, and TGF- $\beta$ 1 levels. The ECP level in polyp tissue homogenate was significantly correlated with the tryptase ( $r=0.58$ ,  $p<0.05$ ; Fig. 5A) and TGF- $\beta$ 1 levels ( $r=0.62$ ,  $p<0.05$ ; Fig. 5C). Tryptase was significantly correlated with the TGF- $\beta$ 1 level ( $r=0.73$ ,  $p<0.05$ ; Fig. 5B).

## DISCUSSION

Inflammatory processes within the mucosa of the upper respiratory tract are believed to play an important role in the development of nasal polyp. Nasal mucosa infiltration by numerous eosinophils and mast cells is the most characteristic feature of nasal polyposis (17). Higher ECP and eosinophil counts were noted in patients with polyposis, suggesting that an excessive

response by activated eosinophils may induce a more profound inflammatory process of the nasal polyp (18). Mast cells act as a key effector in allergic reactions with an ability to release both tryptase and cytokines in response to allergen (19). Nasal polyp has tryptase-containing mast cells in both epithelial and stromal layers, suggesting that activated mast cells which degranulate and release tryptase could participate in nasal polyp generation (17). Another previous study demonstrated that activated mast cells induced the release of eosinophil cationic protein (20). In this study, tryptase was detected and significantly correlated with ECP in nasal polyp tissue, which was responsible for chronic inflammatory response of nasal polyps.

A previous study demonstrated the presence of elevated levels of pro- and active form of MMP-9 in nasal polyps by immunohistochemistry and zymography, while MMP-2 expression remained almost equivalent to both nasal polyp tissue and control mucosa (8). In this study, however, MMP-2 was the predominant form of MMPs in nasal polyp tissue, followed by MMP-9. MMP-9 was reported in an *in vitro* system to play a crucial role in the transmigration of eosinophils (21), and to be overexpressed by eosinophils accumulating in airway walls of asthmatics (10). After eosinophils migrated to nasal mucosa

by their own MMP-2 and -9, they would trigger the synthesis of MMP-2 and -9, which may be involved in the development and maintenance of airway inflammation in asthmatics. Tryptase, which is the specific and major enzyme of human mast cell granules (22), degrades fibronectin and cleaves pro MMP-2 to the activated form (23). Recently, an in vitro study demonstrated that human cultured mast cells could express MMP-9 (11). As shown in the previous study (11, 23), mast cells localized in nasal mucosa expressed MMP-2 and MMP-9 in pathological condition and these findings support the view that the mast cells may influence ECM degradation and remodeling in allergic inflammation. In this study, MMP-2 and MMP-9 were significantly correlated with ECP as well as tryptase, which suggests the possibility that MMP-2 and MMP-9 may be involved in migration of eosinophils and mast cells into nasal polyp tissue. This data also suggests that mast cells and eosinophils might be, either directly or indirectly, responsible for the production of MMPs. MMP-2 may not only be synthesized abundantly in inflamed nasal mucosa, whether they are pro- or active form, but it may also play a role in nasal polyp formation and growth.

Recently, MMP-9 and TIMP-1 expressions were reported in the epithelial cells of nasal mucosa and infiltrating eosinophils as well as bronchial tissue in asthmatics (7, 24, 25). The above data demonstrated that the extent of MMP-9 expression was greater than that of TIMP-1. Moreover, MMP-9 expression significantly correlated with eosinophil infiltration in nasal mucosa. In healthy subjects, MMP-9 was co-secreted with TIMP-1 in 1:1 stoichiometry, and previous reports showed the imbalance between MMP-9 and TIMP-1 in airway inflammation (25, 26). In this study, the MMP-9 level was higher than the TIMP-1 level and significantly correlated with the TIMP-1 level. However, the TIMP-1 level or the molar ratio of MMP-9/TIMP-1 was not different according to clinical parameters, and it was not correlated with cellular activation markers. This result suggests that TIMP-1 is co-secreted with MMP-9 in nasal polyp tissue, and the MMP-9/TIMP-1 imbalance may play a role in regulating inflammatory response in nasal polyp tissue.

Recent studies demonstrated that TGF- $\beta$ 1 was strongly expressed in inflammatory nasal mucosa and allergic rhinitis tissues but not in normal nasal mucosa, and also found that eosinophils represented a major source of TGF- $\beta$ 1 in nasal polyp tissue (13, 27). Recently, Sehgal and Thompson (14) found that TGF- $\beta$ 1 stimulates the MMP-2 and MMP-9 secretory activity in human prostate cell lines. Another in vitro study showed that MMP-9, as well as MMP-2, proteolytically cleave latent TGF- $\beta$ , providing a novel and potentially important mechanism for TGF- $\beta$  activation (28). Furthermore, in patients with chronic airway inflammation, TGF- $\beta$ 1 correlated with the number of macrophage and mast cells in bronchiolar epithelium (29). In nasal polyposis, especially mature nasal polyp, mast cells are more abundant in the submucosa, and they degranulate in human mature nasal polyps (30). Our data

also showed that the TGF- $\beta$ 1 level in nasal polyp tissue was significantly correlated with MMP-2, MMP-9, ECP, and tryptase. According to the above results, we suggest the hypothesis that TGF- $\beta$ 1, in conjunction with MMP-2 and MMP-9, might be involved in ECM degradation and fibrosis in nasal polyp tissue where eosinophils and mast cells are the most prevalent cells.

In conclusion, this study has identified the presence of MMP-2, MMP-9, and TGF- $\beta$ 1 in nasal polyp tissue in relation to eosinophil and mast cell activation. Both MMP-2 and MMP-9 may play a role in migration of eosinophils and mast cells into nasal polyp tissue. Further studies will be needed to elucidate the role of TGF- $\beta$ 1 in nasal polyp inflammation.

## REFERENCES

1. Stiern PLE. *Nasal polyps: relationship to infection and inflammation*. In: Settipane GA, Lund VJ, Berstein JM, Tos M, eds. *Nasal Polyps: Epidemiology, Pathogenesis and Treatment*. OceanSide Publications; Providence, RI; 1997: 119-26.
2. Hamilos DL. *Nasal polyps as immunoreactive tissue*. *Allergy Asthma Proc* 1996; 17: 293-6.
3. Stoop AE, van der Heijden HA, Biewenga J, van der Baan S. *Lymphocytes and non-lymphoid cells in human nasal polyps*. *J Allergy Clin Immunol* 1991; 87: 470-5.
4. Kawabori S, Denburg JA, Schwarz LB, Irani AA, Wong D, Jordana G, Evans S, Dolovich J. *Histochemical and immunohistochemical characteristic of mast cells in nasal polyps*. *Am J Respir Cell Mol Biol* 1992; 6: 37-43.
5. Djukanovic R, Wilson JW, Britten KM, Wilson SJ, Walls AF, Roche WR, Howarth PH, Holgate ST. *Quantitation of mast cells and eosinophils in the bronchial mucosa of symptomatic atopic asthmatics and healthy control subjects using immunohistochemistry*. *Am Rev Respir Dis* 1990; 142: 863-71.
6. Norlander T, Westrin KM, Fukami M, Stiern P, Carlsoo B. *Experimentally induced polyps in the sinus mucosa: a structural analysis of the initial stages*. *Laryngoscope* 1996; 106: 196-203.
7. Hoshino M, Takahashi M, Takai Y, Sim J. *Inhaled corticosteroids decrease subepithelial collagen deposition by modulation of the balance between matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 expression in asthma*. *J Allergy Clin Immunol* 1999; 104: 356-63.
8. Lechapt-Zalcman E, Coste A, d'Ortho MP, Frisdal E, Harf A, Lafuma C, Escudier E. *Increased expression of matrix metalloproteinase-9 in nasal polyps*. *J Pathol* 2001; 193: 233-41.
9. Nagase H. *Activation mechanisms of matrix metalloproteinase*. *Biol Chem* 1997; 378: 151-60.
10. Ohno I, Ohtani H, Nitta Y, Suzuki J, Hoshi H, Honma M, Isoyama S, Tanno Y, Tamura G, Yamauchi K, Nagura H, Shirato K. *Eosinophils as a source of matrix metalloproteinase-9 in asthmatic airway inflammation*. *Am J Respir Cell Mol Biol* 1997; 16: 212-9.
11. Kanbe N, Tanaka A, Kanbe M, Itakura A, Kurosawa M, Matsuda H. *Human mast cells produce matrix metalloproteinase 9*. *Eur J Immunol*

- 1999; 29: 2645-9.
12. Fang KC, Wolters PJ, Steinhoff M, Bidgol A, Blount JL, Caughey GH. *Mast cell expression of gelatinase A and B is regulated by kit ligand and TGF- $\beta$ 1*. *J Immunol* 1999; 162: 5528-35.
  13. Elovic A, Wong DT, Weller PF, Matossian K, Galli SJ. *Expression of transforming growth factor- $\alpha$  and  $\beta$ 1 messenger RNA and product by eosinophils in nasal polyps*. *J Allergy Clin Immunol* 1994; 93: 864-9.
  14. Sehgal I, Thompson TC. *Novel regulation of type IV collagenase (matrix metallo proteinase-9 and -2) activities by transforming growth factor  $\beta$ 1 in human prostate cancer cell lines*. *Mol Biol Cell* 1999; 10: 407-16.
  15. American Thoracic Society. *Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma*. *Am Rev Respir Dis* 1987; 136: 225-44.
  16. Rhichie RF, Alper CA, Graves J, Pearson N, Larson C. *Automated quantitation of proteins in serum and other biologic fluids*. *Am J Clin Pathol* 1973; 59: 151-9.
  17. Park HS, Nahm DH, Park K, Suh KS, Yim HE. *Immunohistochemical characterization of cellular infiltrate in nasal polyp from aspirin-sensitive asthmatic patients*. *Ann Allergy Asthma Immunol* 1998; 81: 219-24.
  18. Keith PK, Conway M, Evans S, Wong DA, Jordana G, Pengelly D, Dolovich J. *Nasal polyps: effects of seasonal allergen exposure*. *J Allergy Clin Immunol* 1994; 93: 567-74.
  19. Redington AE, Howarth PH. *Mast cells, cytokines and asthma*. *Can Respir Dis* 1994; 1: 1-9.
  20. Okayama Y, Kobayashi H, Ashman LK, Holgate ST, Church MK, Mori M. *Activation of eosinophil with cytokines produced by lung mast cells*. *Int Arch Allergy Immunol* 1997; 114(Suppl 1): 75-7.
  21. Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. *Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9*. *Am J Respir Cell Mol Biol* 1997; 17: 519-28.
  22. Broide DH, Lotz M, Cuomo AJ, Coburn DA, Federman EC, Wasserman SI. *Cytokines in symptomatic asthma airways*. *J Allergy Clin Immunol* 1992; 89: 958-67.
  23. Schwartz LB, Bradford TR, Irani AM, Dblouis G, Craig SS. *The major enzymes of human mast cell secretory granules*. *Am Rev Respir Dis* 1987; 135: 1186-9.
  24. Lee HM, Choi JH, Choi CS, Hwang SJ, Lee SH. *Expression of MMP-9 and TIMP-1 in the nasal mucosa of allergic rhinitis*. *Korean J Otolaryngol* 2000; 43: 604-9.
  25. Mautino G, Capony F, Bousquet J, Vignola AM. *Balance in asthma between matrix metalloproteinases and their inhibitors*. *J Allergy Clin Immunol* 1999; 104: 530-3.
  26. Lemjabbar H, Gosset P, Lamblin C, Tillie I, Hartmann D, Wallaert B, Tonnel AB, Lafuma C. *Contribution of 92 kDa gelatinase/type IV collagenase in bronchial inflammation during status asthmaticus*. *Am J Respir Crit Care Med* 1999; 159: 1298-307.
  27. Mautino G, Henriquet C, Jaffuel D, Bousquet J, Capony F. *Tissue inhibitor of metalloproteinase-1 levels in bronchoalveolar fluid from asthmatic subjects*. *Am J Respir Crit Care Med* 1999; 160: 324-30.
  28. Yu Q, Stamenkovic I. *Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF- $\beta$  and promotes tumor invasion and angiogenesis*. *Genes Dev* 1999; 14: 163-76.
  29. de Boer WI, van Schadewijk A, Sont JK, Sharma HS, Stolk J, Hiemstra PS, van Krieken JH. *Transforming growth factor  $\beta$ 1 and recruitment of macrophage and mast cells in airways in chronic obstructive pulmonary disease*. *Am J Respir Crit Care Med* 1998; 158: 1951-7.
  30. Drake-Lee A, Price J. *Mast cell ultrastructure in the inferior turbinate and stroma of nasal polyps*. *J Laryngol Otol* 1997; 111: 340-5.