



Effect of eosinophils activated with *Alternaria* on the production of extracellular matrix from nasal fibroblasts

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ABSTRACT

Background: Eosinophils and fibroblasts are known to play major roles in the pathogenesis of nasal polyps. Fungi are commonly found in nasal secretion and are associated with airway inflammation.

Objective: To investigate whether activated eosinophils by airborne fungi can influence the production of extracellular matrix (ECM) from nasal fibroblasts.

Methods: Inferior turbinate and nasal polyp fibroblasts were stimulated with *Alternaria* or *Aspergillus*, respectively, for 24 hours and ECM messenger RNA (mRNA) and protein expressions were measured. Eosinophils isolated from healthy volunteers were stimulated with *Alternaria* or *Aspergillus* for 4 hours then superoxide, eosinophil peroxidase, and transforming growth factor β 1 were measured. Then activated eosinophils were cocultured with nasal fibroblasts for 24 hours, and ECM mRNA expressions were measured.

Results: *Alternaria* strongly enhanced ECM mRNA expression and protein production from nasal fibroblasts. *Alternaria* also induced the production of superoxide, eosinophil peroxidase, and transforming growth factor β 1 from eosinophils, and activated eosinophils enhanced ECM mRNA expression when they were cocultured without the Transwell insert system.

Conclusions: Eosinophils activated with *Alternaria* enhanced ECM mRNA expression from nasal polyp fibroblasts. *Alternaria* plays an important role in tissue fibrosis in the pathogenesis of nasal polyps by directly or indirectly influencing the production of ECM from nasal fibroblasts.

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Introduction

Nasal polyps are edematous projections of mucosa with a chronic inflammation of the nasal and paranasal sinus mucosa. Noninfectious inflammation plays an important role in the pathogenesis of chronic rhinosinusitis (CRS) with or without nasal polyps. The histologic hallmark of CRS with polyp is marked eosinophilia with an integrated process that involves the mucosal epithelium, extracellular matrix (ECM), inflammatory cells, and their chemical mediators.^{1,2} The formation and growth of nasal polyps require ECM accumulation, and fibroblasts are the main source of ECM.³ Myofibroblasts are an activated cell phenotype of fibroblasts with a high capacity for ECM production.⁴

Eosinophils have been considered as end-stage cells involved in parasite infection and T_H2 -type inflammation. However, eosinophils are now considered as multifunctional leukocytes involved in tissue homeostasis, modulation of adaptive, and innate immune response.⁵ These eosinophils likely play a major role in the

pathophysiology of CRS via the release of their cytotoxic granule proteins, such as eosinophil cationic protein and major basic protein. Eosinophils also produce a variety of inflammatory mediators, such as cytokines, chemokines, lipid mediators, and reactive oxygen species. Eosinophils enhance the inflammatory process by contributing to stromal fibrosis, epithelial damage, increased interstitial edema, and increased production of ECM.⁶

Fungi are ubiquitous in nature, and these airborne fungi and their products may contribute to the development and exacerbation of airway diseases. Fungi are being increasingly recognized as important pathogens in patients with sinusitis, but their role in the pathogenesis of CRS remains controversial. Fungal elements, such as proteins and enzymes, induce inflammatory response and morphologic change of mucosal barriers.⁷ Fungal components stimulate eosinophils through a G protein–dependent mechanism, leading to cellular activation.⁸ Transforming growth factor β (TGF- β), produced by eosinophils, induces myofibroblast differentiation and is involved in the pathogenesis of a variety of airway diseases.⁹ Fibroblasts and eosinophils are major structural and inflammatory cells in CRS with polyps; however, the interaction between activated eosinophils and nasal fibroblasts is not commonly studied. In this study, we investigate whether activated eosinophils by airborne fungi can influence the production of ECM from nasal fibroblasts.

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Methods

Stimulation of Nasal Fibroblast With Fungi

Primary nasal fibroblasts were isolated from 8 patients with CRS with nasal polyps, which were removed during endoscopic sinus surgery, and from the inferior turbinate of 5 patients with septal deviation treated with septal surgery. The diagnosis of CRS with nasal polyps was made according to the European Position Paper on rhinosinusitis and nasal polyps.¹⁰ Individuals were excluded if they had allergy or asthma, had received systemic or topical steroids, or had taken antibiotics or other medications during the 4 weeks preceding the study. Allergy status was defined using the skin prick test or multiple allergen simultaneous test–chemiluminescent assays. The study was approved by the institutional review board of Daegu Catholic University Medical Center. Each participant signed a consent form that outlined the objectives of the research and experiments.

Specimens were aseptically collected, cut into 0.5-mm fragments, and cultured in Dulbecco modified Eagle medium F-12 (Gibco, Grand Island, New York) that contained 10% fetal bovine serum, penicillin at 100 U/mL, streptomycin at 100 µg/mL, and amphotericin B at 1.5 µg/mL at 37°C and 5% carbon dioxide. In this study, only the third to fifth passages were used for experiments. The fibroblasts were incubated with endotoxin removed *Alternaria alternate* and *Aspergillus fumigatus* (Greer Laboratories, Lenoir, North Carolina). After 8, 24, and 48 hours of stimulation, the cells were harvested and stored at –70°C until they were assayed. *Alternaria* and *Aspergillus* were used because these are common pathogens in nasal secretions and respiratory tract diseases. The maximal chemical mediator production was achieved at 50 and 100 µg/mL of *Alternaria* with 8 to 48 hours stimulation.¹¹

Activation of Eosinophils With Fungi

Eosinophils were isolated from peripheral blood obtained from healthy volunteers after they provided informed consent, which was approved by the institutional review board of Daegu Catholic University Medical Center. Isolation was performed according to the previously described method, with minor modifications.¹² Briefly, heparinized venous blood was layered onto 1,085 g/mL of Percoll made in piperazine-N,N'-bis(2-ethanesulfonic acid) (PIPES) buffer (Sigma Chemical, St Louis, Missouri) and centrifuged at 1,000g for 30 minutes. The granulocyte pellet was mixed with anti-CD16 antibody conjugated with magnetic particles (Miltenyi Biotec, Sunnyvale, California) and incubated for 30 minutes. Resuspended cells were separated with a magnetic cell separation system. After the cells were eluted with PIPES buffer, the eosinophil counts and purity were determined. The purity of the eosinophils determined by Randolph staining was greater than 95%. Eosinophils were stimulated for 4 hours with 100, 50 µg/mL of *Alternaria alternate* and 50, 25 µg/mL of *Aspergillus fumigatus* culture filtrate extracts (Greer Laboratories), and 2 ng/mL of phorbol 12-myristate 12-acetate (PMA) was used as positive control.

The generation of superoxide was measured by superoxide dismutase–inhibitable reduction of cytochrome C for 4 hours, and supernatants were collected and stored at –20°C until assay for eosinophil peroxidase (EPO) and TGF-β1. The concentration of EPO (Cell Technology, Fremont, California) and TGF-β1 (R&D System, Minneapolis, Minnesota) were measured with a commercially available enzyme-linked immunosorbent assay kit.

Coculture of Nasal Fibroblasts With Activated Eosinophils

To determine whether physical contact between eosinophils and fibroblasts or the soluble components produced from eosinophils influence the production of ECM, nasal fibroblasts were cocultured with activated eosinophils with or without a Transwell

insert system (Corning Costar, Cambridge, Massachusetts). Eosinophils were activated with 100 µg/mL of *Alternaria*, 50 µg/mL of *Aspergillus*, or 2 ng/mL of PMA for 3 hours. Activated eosinophils were centrifuged at 320g for 10 minutes then cocultured with nasal fibroblasts for 24 hours. One hundred microliters of eosinophils (1×10^6 /mL) was added to fibroblasts or the upper chamber in which insert with porous bottoms (0.4-µm pore size) served as the upper chamber and ordinary tissue culture plate wells as the lower chamber. Nonadhered eosinophils were gently washed 3 times with phosphate-buffered saline, then fibroblasts were harvested for further study.

Real-Time Reverse Transcription–Polymerase Chain Reaction for ECM and α-Smooth Muscle Actin From Nasal Fibroblasts

Total tissue RNA was extracted with Trizol (Invitrogen, Carlsbad, California) according to the manufacturer's instructions. For real-time reverse transcription–polymerase chain reaction (RT-PCR), 1 µg of total RNA was reverse transcribed to complementary DNA (cDNA) using SuPrimeScript RT Premix (Genetbio Inc, Daejeon, Korea) following the manufacturer's protocols. Quantitative PCR was then performed in a mini Opticon system (Bio-Rad Laboratories, Hercules, California) using a 1-µL cDNA template, 1 nmol of primers, and 10 µL of SYBR premix ExTaq (Takara, Kyoto, Japan) in a total volume of 20 µL of reaction. The forward and reverse primers were as follows: β-actin, 5-ACAGGAAGTCCCTTGCCATC-3 and 5-AGGGAGACCAAAAG CCTTCA-3; α-smooth muscle actin (α-SMA), 5-ATAGAACATGGCA TCATCACCAC-3 and 5-GGGCAACGGAAGCTCATTGTA-3; fibronectin, 5-GCCAGATGATGAGCTGCAC-3 and 5-GAGCAATGGCA CCGAGATA-3; tissue inhibitors of matrix metalloproteinases 1 (TIMP-1), 5-CCTTATACCAGCGTTATGAGATCAA-3 and 5-AGTGATG TGCAAGAGTCCATCC-3; and matrix metalloproteinase 9 (MMP-9), 5-ATTTCTGCCAGGACCGTTCTACT-3 and 5-CAGTTTGATCCG GCAAAGTGGCT-3. The cDNA was amplified with initial denaturation at 95°C for 10 minutes, followed by PCR of 40 cycles of 95°C for 5 seconds and 58°C for 30 seconds, and finally 1 cycle of melting curve after cooling at 60°C for 60 seconds. To confirm amplification specificity, the PCR products from each primer pair were subjected to a melting curve analysis. Analysis of relative gene expression was performed by evaluating quantitative RT-PCR data by the $2^{-\Delta\Delta Ct}$ method. Each experiment was repeated at least 3 times, and GAPDH was used as a housekeeping gene for internal control. The levels of gene expression were determined by normalizing relative to β-actin expression.

Western Blot Analysis of ECM and α-SMA From Nasal Fibroblasts

Nasal fibroblasts were lysed using radioimmunoprecipitation assay buffer (Pierce, Rockford, Illinois). The lysates were subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto NC membranes (Bio-Rad, Berkeley, California). Membranes were blocked with membrane blocking solution (Invitrogen, Carlsbad, California) and were incubated with the antibodies, such as α-SMA (Abcam, Cambridge, Massachusetts), MMP-9 (Cell Signaling, Beverly, Massachusetts), collagen type 1, fibronectin, TIMP-1, and glyceraldehyde 3-phosphate dehydrogenase (Santa Cruz Biotechnology, Santa Cruz, California). After being incubated for 1 hour, the membranes were washed and then treated with peroxidase-conjugated anti-rabbit IgG (Santa Cruz Biotechnology). Bands were visualized with horseradish peroxidase–conjugated secondary antibodies and an ECL system (Pierce, Rockford, Illinois). The band densities were measured using the multi Gauge version 2.02 (Fujifilm, Tokyo, Japan). The band intensities were expressed as a percentage of treated vs untreated cells.

Statistical Analysis

All experiments were performed at least 5 times and revealed comparable results. The results are presented as mean (SD). The statistical difference between groups was assessed by Wilcoxon signed-rank test and Kruskal-Wallis 1-way analysis of variance (SPSS version 18.0; SPSS Inc, Chicago, Illinois). $P \leq .05$ was considered significant.

Results

Production of ECM and α -SMA mRNA and Protein Expression From Nasal Fibroblast With Fungi

To determine the optimal concentration and time for the maximal expression of mRNA for ECM and α -SMA, a kinetic study was performed for 48 hours. When the nasal fibroblasts were stimulated with 100 μ g/mL of *Alternaria* for 24 hours, collagen type 1, TIMP-1, MMP-9, and α -SMA mRNA expressions were highest compared with the other times and concentrations (Fig 1). When the nasal polyp fibroblasts were stimulated with *Aspergillus*, only MMP-9 mRNA expression was increased. When the nasal fibroblasts from the patients with septal deviation were stimulated with fungi, mRNA expression was much weaker than nasal polyp fibroblasts; however, mRNA expression pattern was very similar (Fig 1). Therefore, further experiments were performed with nasal polyp fibroblasts.

ECM and α -SMA protein expressions within the nasal polyp fibroblasts were determined with Western blot. After 24-hour

stimulation of nasal polyp fibroblasts with *Alternaria* and *Aspergillus*, fibronectin, TIMP-1, and MMP-9 protein expressions were significantly increased with *Alternaria*. Only fibronectin protein expression was increased with *Aspergillus* (Fig 2).

Activation of Eosinophils With Fungi

Eosinophils were stimulated with fungi for 4 hours. Maximal superoxide production was achieved 3 hours after stimulation with fungi, and *Alternaria* (1.0 [0.7] nmol/L with 50 μ g/mL) induced the production of superoxide stronger than *Aspergillus* (0.7 [0.3] nmol/L with 50 μ g/mL) (Fig 3).

We detected EPO and TGF- β 1 production from eosinophils with the enzyme-linked immunosorbent assay method. EPO production was significantly increased when eosinophils were stimulated with fungi (33.3 [13.9] mU/mL with 50 μ g/mL and 56.3 [16.7] mU/mL with 100 μ g/mL of *Alternaria*) than the nonstimulated one. TGF- β 1 production from eosinophils was significantly increased with *Alternaria* (31.8 [10.6] pg/mL with 50 μ g/mL and 39.5 [12.7] pg/mL with 100 μ g/mL of *Alternaria*) but not with *Aspergillus* (Fig 3).

Production of ECM and α -SMA mRNA From Nasal Fibroblast When Cocultured With Activated Eosinophils

On the basis of the results of eosinophil activation and ECM mRNA expression with fungi, eosinophils were stimulated with 100 μ g/mL of *Alternaria* and 50 μ g/mL of *Aspergillus* and then cocultured with nasal polyp fibroblasts for 24 hours.

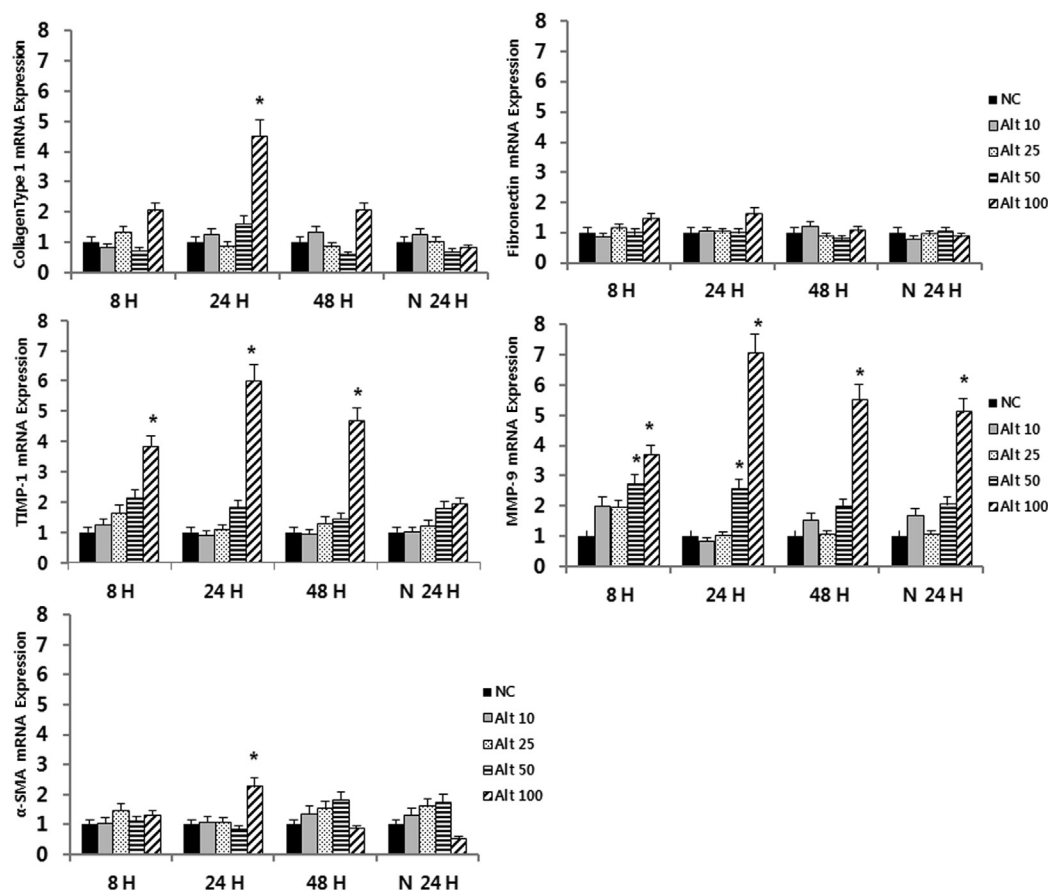


Figure 1. The effect of *Alternaria* on the expression of extracellular matrix (ECM) messenger RNA (mRNA) from nasal fibroblasts. ECM mRNA expressions were measured after 8, 24, and 48 hours stimulation with fungi. When the nasal fibroblasts were stimulated with 100 μ g/mL of *Alternaria* for 24 hours, mRNA expression was highest compared with the other concentrations. Data are expressed as mean (SD) and are representative of 5 independent experiments. * $P < .05$ compared with the nonstimulated fibroblasts. α -SMA indicates α -smooth muscle actin; Alt 10, 25, 50, and 100, 10, 25, 50, and 100 μ g/mL of *Alternaria*; MMP-9, matrix metalloproteinase 9; N 24 h, normal inferior turbinate fibroblasts stimulated for 24 hours; NC, negative control; TIMP-1, tissue inhibitors of matrix metalloproteinases 1.

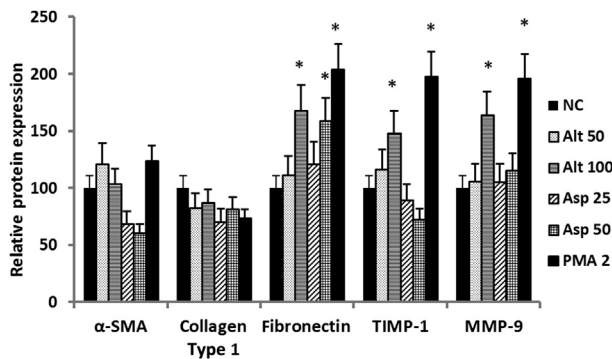


Figure 2. The effect of *Alternaria* and *Aspergillus* on the production of extracellular matrix (ECM) proteins from nasal fibroblasts. ECM protein production after 24-hour stimulation with fungi. Fibronectin, tissue inhibitors of matrix metalloproteinases 1 (TIMP-1), and matrix metalloproteinase 9 (MMP-9) protein expressions were significantly increased by *Alternaria*. In addition, only fibronectin protein expression was increased by *Aspergillus*. Data are expressed as mean (SD) and are representative of 5 independent experiments. * $P < .05$ compared with the nonstimulated fibroblasts. α-SMA indicates α-smooth muscle actin; Alt 50 and 100, 50 and 100 μg/mL of *Alternaria*; Asp 25 and 50, 25 and 50 μg/mL of *Aspergillus*; MMP-9, matrix metalloproteinase 9; NC, negative control; PMA 2, 2 ng/mL of phorbol 12-myristate 12-acetate; and TIMP-1, tissue inhibitors of matrix metalloproteinases 1.

To determine whether the soluble components produced from eosinophils or physical contact between fibroblasts and eosinophils play a role in the production of ECM, fibroblasts and eosinophils were cocultured with or without the Transwell insert system. When the nasal fibroblasts were cocultured with activated eosinophils with 100 μg/mL of *Alternaria* without the Transwell insert system, collagen type 1, TIMP-1, MMP-9, and α-SMA mRNA expressions were significantly higher than fibroblasts, which were cocultured with nonstimulated eosinophils and cocultured with a Transwell insert system. Fibronectin mRNA expression did not increase with coculture of nasal fibroblasts and activated eosinophils. Unlike cocultures using activated eosinophils with *Alternaria*, the activated eosinophils with 50 μg/mL of *Aspergillus* did not influence the expression of ECM mRNA from nasal fibroblasts (Fig 4).

Discussion

A variety of inflammatory cells infiltrations, including lymphocytes, neutrophils, eosinophils, and mast cells, are characteristic histologic findings of CRS with or without nasal polyps. Squamous metaplasia and basement membrane thickening with the enhanced accumulation of fibronectin and subepithelial collagen deposition

with fibrosis are characteristic findings of tissue remodeling in CRS.¹³ Both the fibroblasts and eosinophils are potential sources of profibrotic cytokines, which contribute to tissue remodeling. In nasal polyps, an increase in MMP-9 has been found with low levels of TIMP-1. MMP-9 in nasal polyps plays an important role in tissue remodeling during polyp development.¹³ Increases in vascularization, α-SMA myofibroblasts, and deposition of collagens are also documented in nasal polyps.¹⁴ We found the production of ECM from nasal polyp fibroblasts by *Alternaria* itself and activated eosinophils with *Alternaria*.

In this study, *Alternaria* strongly induces fibronectin, TIMP-1, and MMP-9 protein production from nasal polyp fibroblasts. In addition, *Aspergillus* only induced the production of fibronectin protein. Most fungi are ubiquitous, and the host immune responses to fungi require maintaining a stable host-fungus association. Toll-like receptor (TLR) 2, TLR4, and TLR9 have been known as the main TLRs that are involved in sensing fungal components. TLR2 and TLR5 are important receptors in inducing the production of chemical mediators from nasal fibroblasts by fungi.¹⁵ The different pathogens or immune cells may induce immune responses through different immune pathways. These differences may be associated with the unique molecular pattern, the peptide specificity of fungal enzymes, the major immune-triggering components of fungi, and the immunologic activity of fungi.¹⁶ This study found that *Alternaria* was a stronger immunostimulant for the production of ECM from nasal fibroblasts. Although we did not evaluate the immune mechanism of ECM production by fungi, TLR or another pattern recognition receptor may interact with antigenic components of fungi. *Alternaria* also enhanced TIMP-1 and MMP-9 mRNA expressions from septal deviated patients' nasal fibroblasts, which reveal a similar pattern with nasal polyp fibroblasts. The expression intensity was much lower with septal deviated patients' nasal fibroblasts, so we used nasal polyp fibroblasts for further studies. In healthy nasal mucosa, fungi cannot enter the tissue and do not strongly interact with mucosal structural cells. However, mechanical or enzymatic injuries and inflammatory conditions may facilitate fungi to directly interact with nasal fibroblasts and enhance local inflammatory responses.

Although the role of eosinophils in nasal polyp pathogenesis remains an area of debate, there has been growing recognition that eosinophils have an important role in polyp formation. Less attention has been given to the role of eosinophils in tissue repair and remodeling in nasal polyps. Eosinophils are a source of several molecules implicated in tissue remodeling with the production of TGF-α, TGF-β1, fibroblast growth factor, and vascular endothelial growth factor, interleukin (IL) 13, and IL-17. These fibrogenic mediators induce a fibrogenic phenotype fibroblast and dysregulate the ECM homeostasis, which led to tissue remodeling and fibrosis.¹⁷

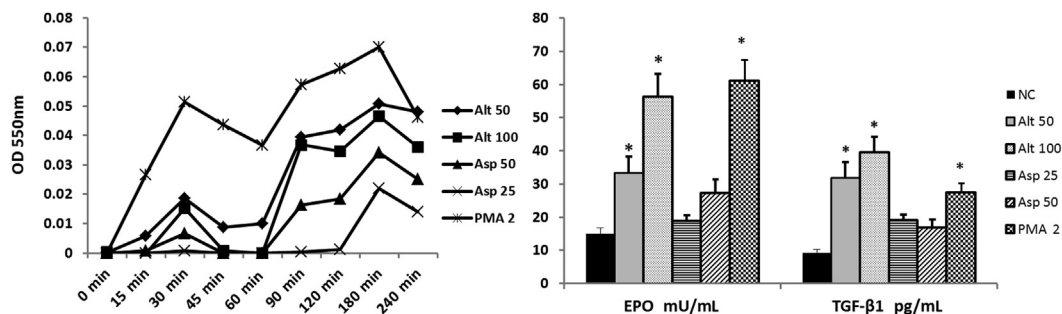


Figure 3. Activation of eosinophils with fungi. Maximal superoxide production was achieved 3 hours after stimulation with fungi, and 100 μg/mL of *Alternaria* induced the maximal eosinophil peroxidase (EPO) and transforming growth factor (TGF) β1 production from eosinophils. Data are expressed as mean (SD) and are representative of 5 independent experiments. * $P < .05$ compared with the nonstimulated eosinophils. α-SMA indicates α-smooth muscle actin; Alt 50 and 100, 50 and 100 μg/mL of *Alternaria*; Asp 25 and 50, 25 and 50 μg/mL of *Aspergillus*; MMP-9, matrix metalloproteinase 9; NC, negative control; PMA 2, 2 ng/mL of phorbol 12-myristate 12-acetate; and TIMP-1, tissue inhibitors of matrix metalloproteinases 1.

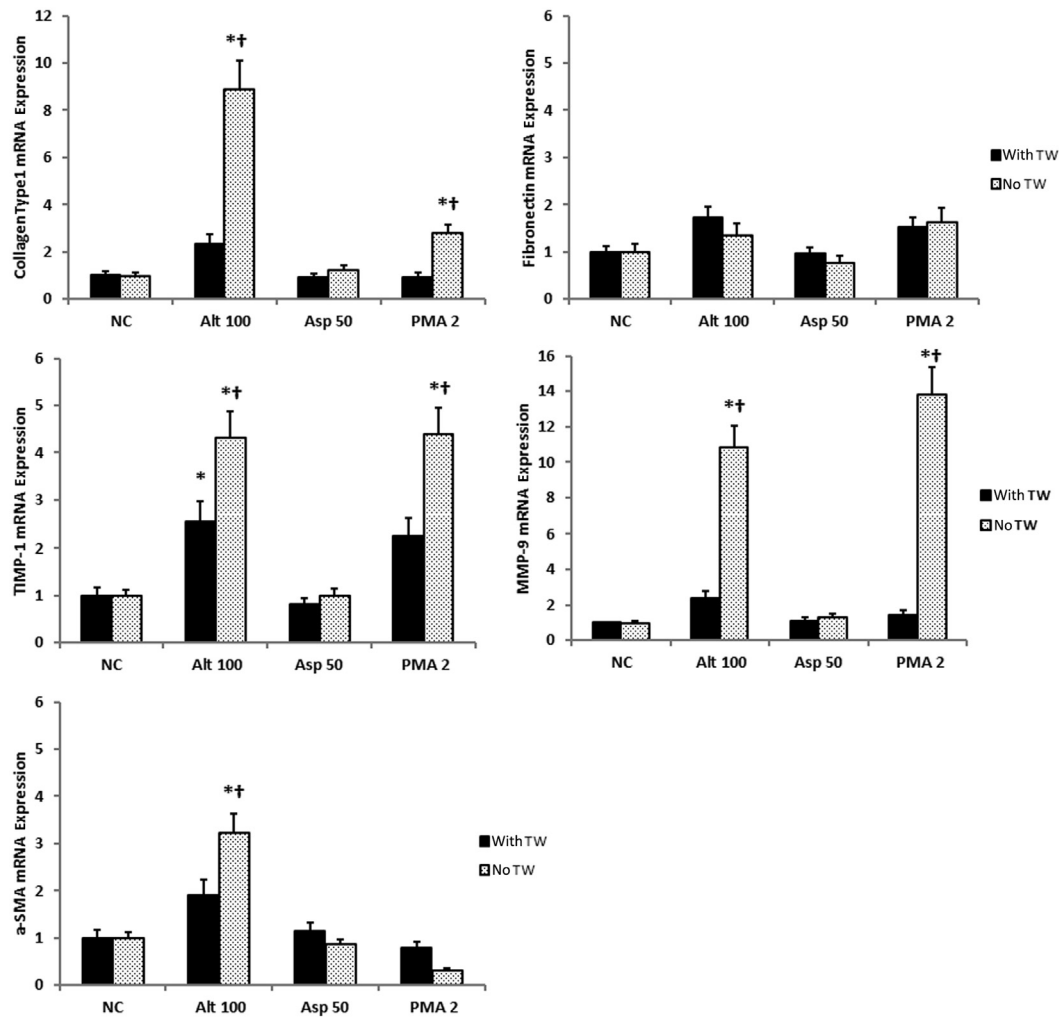


Figure 4. Expression of extracellular matrix messenger RNA (mRNA) from nasal polyp fibroblasts when cocultured with activated eosinophils by fungi. Eosinophils were cocultured with fibroblast with or without a Transwell insert system (TW) for 24 hours. When the activated eosinophils were cocultured without the TW, collagen type 1, tissue inhibitors of matrix metalloproteinases 1 (TIMP-1), and matrix metalloproteinase 9 (MMP-9) mRNA expressions from fibroblasts were significantly higher than those that were cocultured with nonstimulated eosinophils and the TW. Data are expressed as mean (SD) and are representative of 5 independent experiments. * $P < .05$ compared with cocultured with nonstimulated eosinophils. † $P < .05$ compared with cocultured with the TW. Alt 100 indicates 100 $\mu\text{g/mL}$ of *Alternaria*; Asp 50, 50 $\mu\text{g/mL}$ of *Aspergillus*; NC, negative control; and PMA 2, 2 ng/mL of phorbol 12-myristate 12-acetate.

Alternaria enhanced EPO and TGF- β 1 production from eosinophils, and when the activated eosinophils were cocultured with nasal fibroblasts, these fibrogenic mediators from eosinophils may enhance the production of ECM from fibroblasts. Fungi can interact with the eosinophils to produce chemical mediators through pattern recognition receptors, such as protease activated receptor. *Alternaria* aspartate protease and other exogenous fungus-derived protease-activating receptor activating enzymes affect the activation of immune cells.¹⁶

The eosinophils were stimulated with fungi and then cocultured with nasal polyp fibroblasts. Without a Transwell insert system, which allows the physical contact between activated eosinophils and nasal fibroblasts, collagen type 1, TIMP-1, MMP-9, α -SMA mRNA expressions were significantly increased compared with inhibited physical contact with a Transwell insert system. If the eosinophils and fibroblasts are stimulated by fungi at the same time, ECM mRNA expression might be stronger. Under stimulation of fungi on both eosinophils and fibroblast simultaneously, the concentration of fibrogenic chemical mediators produced by eosinophils is expected to be much higher. However, we pre-treated eosinophils with fungi to eliminate the direct effect of

fungi to the fibroblasts. Eosinophils adhered to fibroblasts stimulated with certain cytokines, such as IL-1 α , TNF- α , and interferon γ . In addition, eosinophils adhered to the fibroblasts with the up-regulated cell surface galectin 9 protein.¹⁸ Considering these findings, we need to confirm the molecular mechanisms of cellular interaction between activated eosinophils and nasal fibroblasts. Although we did not evaluate the expression of eosinophil cell surface adhesion molecules, airborne allergens can enhance the β_2 -integrin and other adhesion molecules.¹⁹ Coculture of foreskin fibroblasts with eosinophil cell line (AML 14.3D10) induced a significant increase in the expression of mRNA for plasminogen activator inhibitor 1, fibronectin, and TIMP-1. Collagen type 1 gene expression was not significantly increased.²⁰ The collagen type 1 gene expression pattern was different between nasal fibroblasts and skin fibroblasts. Human fibroblasts are not a homogenous population and they express different structural and functional features that are dependent on their location within the body.²¹ The anatomical locations and pathologic conditions determine the characteristics and phenotypes of fibroblasts. Because of the heterogeneous nature of the fibroblasts, further studies are needed to fully understand the inflammatory

cell interactions and remodeling process in the pathogenesis of nasal polyps.

Fibroblasts are involved in the pathogenesis of nasal polyps by the accumulation of ECM with increasing the proliferation of fibroblasts. In addition, eosinophils and fibroblasts are known to play major roles in the pathogenesis of nasal polyps. In this study, we found that *Alternaria* directly induces ECM mRNA expression and protein production from nasal fibroblasts. Airborne fungi cannot directly interact with the fibroblasts and eosinophils in the nasal mucosa. If mucosal epithelial cells were damaged by physical trauma or enzymatic activities of pathogens, fungal allergens can interact with structural cells or immune cells, enhancing the development airway inflammation, remodeling, and nasal polyp formation. We did not analyze the ECM protein expression because too much blood was needed to get enough eosinophils for coculture with fibroblasts and therefore eosinophils activated with *Alternaria* enhanced ECM mRNA expression from nasal fibroblasts. The present experiments suggest that *Alternaria* plays an important role in tissue fibrosis in the pathogenesis of nasal polyps not only by directly stimulating the nasal fibroblasts but also by indirectly influencing the production of ECM from nasal fibroblasts by the activation of eosinophils.

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