German Cockroach Extract Induces Matrix Metalloproteinase 1 Expression, Leading to Tight Junction Disruption in the Airway Epithelial Cells

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Directed by Professor Myung Hyun Sohn

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ABSTRACT

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Background Cockroach exposure is a pivotal cause of asthma, and the barrier function of the airway epithelium was shown to be impaired in this disease. Tight junctions are intercellular structures, required for epithelial barrier function maintenance. Matrix metalloproteinases (MMPs) digest extracellular matrix components and are involved in asthma pathogenesis; MMP1 is a collagenase with a direct activity in the airway obstruction in asthmatics.

Objective To investigate the mechanisms of MMP1 expression by German cockroach extract (GCE) and whether MMP1 release alters cellular tight junctions in the airway epithelial cells.

Methods Human airway epithelial cells (H292) were treated with GCE. mRNA and protein levels were determined using real-time polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA),

respectively. Tight junction proteins were detected using immunofluorescence staining. Epithelial barrier function was measured by transepithelial electrical resistance (TEER). GM6001 as a potent MMP inhibitor, PD98059 as a MAPK/ERK kinase inhibitor, siRNAs against MMP1, ETS1, and SP1, and anti-TLR2 antibody were used as pre-treatments prior to the GCE stimulation. The levels of tight junction proteins and ERK phosphorylation were determined using western blotting.

Results GCE was shown to increase MMP1 expression, tight junction protein degradation, and decrease TEER. GM6001 treatment and transient cell transfection with MMP1 siRNA inhibited GCE-induced tight junction disruption. Additionally, transient transfection using ETS1/SP1-targeting siRNA and anti-TLR2 antibody pretreatment prevented MMP1 expression inhibition and tight junction degradation. PD98059 effectively blocked MMP1 release, ETS1/SP1 expression, and tight junction alteration.

Conclusions This study demonstrated that GCE treatment induces MMP1 expression, leading to tight junction disruption, and elucidated MMP1 transcriptional regulation in the airway epithelial cells. These findings may help develop novel therapeutic strategies for the treatment of airway diseases.

Key words: german cockroach, asthma, matrix metalloproteinase, airway epithelial cell, tight junction

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I. INTRODUCTION

Asthma is a major health concern in all age groups, and the allergic form of asthma occurs due to a wide range of factors, including molds, pet dander, cockroaches, and ragweed[1,2]. Early-life exposure to and sensitization with various allergens, including cockroaches and *Alternaria,* were demonstrated in children with severe forms of asthma[3,4]. Cockroaches produce several allergens, and the exposure to the increased levels of these allergens represents a major risk factor in the sensitized individuals[5,6]. Among children who live in inner-city area, the highest levels of morbidity due to asthma were associated with the presence of both a positive skin-test response to

cockroach allergen and current exposure to high levels of cockroach allergen in the bedroom[4]. Cockroaches are found throughout the environment and both the German cockroach (*Blattella germanica*) and the American cockroach (*Periplaneta americana*) have been associated with asthma. *B. germanica* is a widely distributed urban pest, which is most commonly found at homes, apartments, restaurants and hotels. *P. americana* often lives outside in sewers, stream tunnels and drainage systems; they can also be found in large commercial buildings such as grocery markets, restaurants and hospitals[6]. Saliva, feces, cast skins and dead bodies from cockroaches are potential sources of allergens. Feces (frass) are believed that the most likely source of cockroach allergens because of the amount of excrement secreted from each cockroach, the potentially high number of cockroaches dwelling in homes and the fact that desiccated frass is likely to crumble and become airborne as dust. Fecal extracts have been shown to contain 3 to 6 times more of the cockroach allergens Bla g 2 and Bla g 1 than cockroach whole body extracts. German cockroach (GC) frass and the commercially available whole body cockroach extract contain serine protease activity[5,6]. Three cockroach species from Korea (*B. germanica*, *P. americana* and *T. fuliginosa*) were confirmed to contain gelatinolytic activity[7]. Previously, it was reported that cockroach allergens have a proteolytic activity, increasing inflammatory cytokines in human airway epithelial cells or a Toll-like receptor (TLR) 2 agonist that directly affects the neutrophils, inducing an early innate immune response[8]. Using neutrophils as an early marker of the innate immune response, neutrophils isolated from the airways following GC frass inhalation express

TLR2 and release cytokines. GC frass directly affected neutrophil cytokine production via TLR2, but not TLR4, as evidenced by the use of TLR-neutralizing antibodies and neutrophils from TLR-deficient mice[8]. Furthermore, it was demonstrated that cockroach frass proteases can cleave pro-matrix metalloproteinase (MMP) 9, which plays a role in the airway remodeling[9].

MMPs are endopeptidases belonging to the metzincin superfamily. Although the MMPs are the products of different genes, similarity in the structural of their modular domains imparts a high degree of homology among the various members. There are three distinct common domains: a zinc-containing catalytic domain, an amino-terminal pro-peptide domain containing a cysteine residue that chelates the zinc ion in the catalytic domain and holds the enzyme in a latent pro-form, and a carboxy-terminal hemapexin-like domain that contributes to substrate recognition[10]. MMP family includes zinc- and calcium-dependent endopeptidase[11], which can degrade a variety of substrates, and they play crucial roles in the regulation of tissue remodeling and regeneration[12]. In human branching morphogenesis, immunohistochemistry detects the expression of MMP1, 9, TIMP1, 2, and TIMP3 in the fetal epithelium and MMP2, MMP1, TIMP2, and TIMP3 in the pulmonary vascular endothelium[13]. The production and activity of MMPs are tightly controlled by transcriptional and post-translational mechanisms in combination with proenzyme activation and inhibition of active MMPs. However, when not tightly regulated, MMPs can become dangerous, and were shown to be involved in cancer metastasis and cardiovascular diseases. Recent studies revealed that MMP dysregulation can be

observed in chronic obstructive pulmonary disease (COPD), asthma, interstitial lung diseases (ILDs), and lung cancer[11]. MMP levels and/or activity in the individuals with asthma were shown to be significantly higher than those in the control subjects[12]. Extensive studies confirmed that MMP expression and activity of MMP1, 2, 3, 9, and 12 are associated with asthma pathogenesis and asthma-associated airway remodeling[11]. Using animal models, the increase in MMP9 activity in the airway mucosa was shown to be associated with epithelial damage, subepithelial basement membrane alterations, and subepithelial collagen deposition[16]. Additionally, epithelium-derived metalloproteinases were shown to be involved in the pathogenesis of asthma[17]. In other studies, while MMP2and MMP9 were not required for induction of Th2 inflammation in response to sensitization and allergen challenge in mice, chemical inhibition of MMPs using GM6001, and or genetic ablation of MMP2, and MMP9 resulted in accumulation of inflammatory cells in the lungs and asphyxiation. The mechanism responsible for accumulation of allergic inflammatory cells in the lung parenchyma in the absence of MMPs includes loss of chemokine gradient formation in the airways that is critical for resolution of inflammation[18,19]. Recent study reported that MMP1 activation was associated with asthma exacerbation severity[20]. In terms of treatment of asthma, a high degree of uncertainty surrounds the therapeutic use of MMP inhibitors. As the substrates of MMPs have diverse biologic effects, use of pharmacological inhibitors in asthma should be approached with caution. Recent evidence also suggests that inhibition of MMPs may promote a deviation toward a Th2-type cytokine profile[10].

The airway epithelium provides a potential protective layer between the internal space of the lung and the external environment. The bronchial epithelium has a pseudostratified structure, with the majority of cells being ciliated columnar epithelial cells, which fulfil a vital role in the clearance of inhaled pathogens and particles by the mucociliary escalator[15]. Early thinking suggested that the bronchial epithelium was merely an inert physical barrier against ingression of inhaled pathogens and toxins. However, it has become apparent that the epithelium also provides a chemical and immunologic barrier between the airway submucosa and the external environment. Crucially, there is clear evidence that these functions may be perturbed in the bronchial epithelium of asthmatic subjects[21]. Under physiological conditions, the epithelium forms a highly regulated and impermeable tight junction-containing barrier. The tight junction is central to the function of the epithelium as a selectively permeable barrier: the ‘gate’ function, acting as a selectively but variably permeable barrier to the passage of ions and solutes via the paracellular pathway, and the ‘fence’ function, acting as a site of demarcation between the apical and basolateral domains of the cells membrane to define epithelial polarity[22]. Tight junctions are composed of transmembrane proteins such as occluding and claudins, which are linked to the actin cytoskeleton by ZO1, ZO2, and, ZO3, and they interact to form a complex protein network[23]. Structural integrity of the epithelium is further maintained through cell-cell and cell–extracellular matrix interactions involving adherens junctions, desmosomes, and hemidesmosones[24]. Disruption of the columnar epithelium enables tissue-damaging agents and infectious particles to penetrate the airway wall, thereby

facilitating toxic, immune, and inflammatory responses with ensuing tissue damage. Several reports demonstrated that the barrier function of airway epithelium is impaired in asthma, which enables the allergen particles to penetrate the airway wall, inducing immune and inflammatory responses and causing tissue damage[24,25]. Compared with normal, epithelial cells brushed from asthmatic airways, cultured in vitro and differentiated on an air liquid interface, are unable to form effective tight junctions fully even though the cells have been passaged several times and separated from any airway inflammatory cells or mediators for 6 to 8 weeks[24]. At this time, measurement of transepithelial resistance across the differentiated epithelial cell cultures at baseline is markedly reduced, indicating increased leakiness. Apical exposure of the asthmatic cultures to injurious agents such as tobacco smoke extract revealed a further reduction in transepithelial resistance at much lower concentrations than required to achieve a similar response in differentiated normal epithelium. As observed in the asthmatic biopsies, whole mount immunofluorescence confocal microscopy with antibodies directed to ZO1 and occludin confirmed poorly developed tight junctions in asthmatic compared with normal cultures. Environmental factors such as respiratory viruses and proteolytically active allergens (eg, *Dermatophagoides pteronyssinus* allergen cysteine protease) have the capacity to disrupt tight junctions further and increase epithelial permeability[24]. One of the most prominent features of asthma is the changed properties of the lung extracellular matrix (ECM) associated with tissue stiffness and elasticity. The composition of the ECM is altered in asthma, mainly by an imbalance in its deposition and degradation.

One important class of ECM-degrading enzymes are MMPs and their inhibitors, and tissue inhibitors of metalloproteinases (TIMPs)[21]. Environmental insults can interfere with the MMP/TIMP balance, so that it has been shown that rhinovirus infection induces MMP9 expression in airway epithelial cells15. MMP9 was shown to modulate tight junction integrity and cell viability directly in the human airway epithelium[26].

In this study, the effects of the GCE on MMPs, and the regulatory processes in the airway epithelium involved in asthma pathogenesis were analyzed. Furthermore, the mechanisms underlying the GCE-associated effects on MMP1 in human airway epithelial cells, and the relationship between the expression and activity levels of these proteins and tight junction integrity were investigated.

II. MATERIALS AND METHODS

1. Reagents

German cockroach extract (GCE) was purchased from the Arthropods of Medical Importance Resource Bank, Yonsei University College of Medicine (Seoul, Korea). The small interfering RNAs (siRNAs) against *AP1*, *NF-κB*, *SP1*, *ETS1*, and *MMP1* were purchased from Santa Cruz Biotechnology (CA, USA). Anti-TLR2 antibody and anti-phosphorylated and control p44/42 antibodies were purchased from R&D

systems (MN, USA) and Cell Signaling Technology (MA, USA), respectively. A broad-spectrum MMP inhibitor, GM6001, and a mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) inhibitor, PD98059, were purchased from Santa Cruz Biotechnology (CA, USA) and Cell Signaling Technology (MA, USA), respectively.

2. Cell culture

Human epithelial carcinoma cells, H292 (ATCC, VA, USA), were plated into 6- or 12-well culture plates in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 100 U/mL penicillin and streptomycin (GE Healthcare Life Sciences, UT, USA).

3. Real-time polymerase chain reaction (PCR)

Total RNA was isolated from cells using TRIzol reagent and cDNA was synthesized using random primers and SuperScript III reverse transcriptase (Invitrogen, CA, USA). Real-Time PCR was performed with AccuPower GreenStar qPCR premix by using ExicyclerTM 96 Real-Time Quantitative Thermal Block (Bioneer, Seoul, Korea) and mRNA expression levels were normalized to those of the housekeeping gene, *GAPDH*. Primers are shown in Table 1.

Table 1. List of qPCR primers used in this study

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
MMP-1	AAAATTACACGCCAGATTTGCC	GGTGTGACATTACTCCAGAGTTG
MMP-9	TGTACCGCTATGGTTACACTCG	GGCAGGGACAGTTGCTTCT
TIMP-1	CTTCTGCAATTCCGACCTCGT	ACGCTGGTATAAGGTGGTCTG
Occludin	AGCAGCGGTGGTAACTTTG	AGTTGTGTAGTCTGTCTCATAGTG
ZO-1	AAACAAGCCAGCAGAGACC	CGCAGACGATGTTCATAGTTTC
Ets-1	GATAGTTGTGATCGCCTCACC	GTCCTCTGAGTCGAAGCTGTC
Sp-1	TTGAAAAAGGAGTTGGTGGC	TGCTGGTTCTGTAAGTTGGG
NF-kB	GCAGATGGCCCATACCTTCA	CACCATGTCCTTGGGTCCAG
AP-1 (c-fos)	AAACCCATCACCATCTTCCA	GTGGTTCACACCCATCACAA

4. Enzyme-linked immunosorbent assay (ELISA)

Human MMP1 and 9 protein levels in culture cell supernatants were determined using the ELISA kits following the manufacturer's instructions (R&D Systems, MN, USA).

5. Western blotting

Following the stimulation with GCE, cells were washed with cold phosphate-buffered saline (PBS) and lysed using M-PER supplemented with

proteases (Thermo Fisher Scientific, MA, USA). Cell lysates were centrifuged at 12,000 rpm for 5 min and the total protein contents were analyzed using Bradford assay (Bio-Rad, CA, USA). Protein extracts were subjected to 3.5-10% SDS-PAGE and western blot analysis with anti-ZO1 and anti-occludin antibodies (Invitrogen, CA, USA). Protein-antibody complexes were observed using the X-ray films and the ECL detection kit (GE Healthcare, Buckinghamshire, UK).

6. Immunofluorescence

Following the exposure to GCE, cells seeded onto the Nunclon Delta Surface (Thermo Fisher Scientific, MA, USA) were washed with PBS, fixed in 4% paraformaldehyde for 20 min at room temperature, incubated successively in 1 mM NH_4Cl and 4% Tween, labeled with primary antibody against ZO1 and occludin for 1 h, and incubated with FITC-labeled goat-anti mouse IgG conjugate (Invitrogen, CA, USA). Cell-antibody complexes were analyzed on an Olympus Fluo FV100 confocal microscope (Olympus Corporation, Tokyo, Japan).

7. Electrical resistance

Transepithelial electrical conductance was monitored dose-dependently using a volt-ohm meter and chopstick electrodes, according to manufacturer's instructions (World Precision Instruments, FL, USA). PBS was applied for each measurement and then removed.

8. siRNA transfection

Cells were transiently transfected with the siRNAs against *NF-κB*, *AP1*, *SP1*, and *ETS1,* and siRNA against *MMP1* by using the TransIT-X2 Dynamic Delivery System (Mirus Bio, WI, USA), and incubated in complete media overnight, following the manufacturer's recommendations. After the GCE treatment, the supernatants were harvested for the MMP1 ELISA, while the cells were prepared for MMP1 qRT-PCR and western blot analysis of tight junction proteins.

9. Statistical analysis

All data are presented as mean ± standard error of the mean (SEM) and differences between groups were analyzed using Student's *t* test. Data were analyzed using SPSS v.20 (IBM Corp, Armonk, NY, USA). $P < 0.05$ was considered statistically significant.

III. RESULTS

1. GCE induces MMP expression in the airway epithelial cells in vitro

To investigate whether GCE can induce MMP expression, since their overproduction was demonstrated in asthmatic patients[15], human airway epithelial cells were

stimulated with GCE (50 μg/mL) for 0-48 h and the cells were harvested to determine *MMP1, 2, 7, 9, 12,* and *TIMP1* expression, and cell culture supernatants to determine MMP levels using ELISA. As shown in Figure 1A, GCE significantly induced only *MMP1* (9.9-fold) and *MMP9* (18.7-fold) expression at 6 h, compared with those in the control (n=3, $P<0.05$). Time-course expression analyses showed that *MMP1* significantly increased at 12 h after the initiation of the treatment (23.2-fold compared with the results at 0 h), while the *MMP9* expression peaked at 6 h and gradually decreased thereafter (Fig. 1B and 1C). Furthermore, as TIMP is considered one of the important endogenous inhibitors of MMPs[15], the expression of *TIMP1* was analyzed as well. However, no correlation between the *MMP1*, *9,* and *TIMP1* expression following the GCE treatment was observed (Fig. 1D). Additionally, MMP1 and MMP9 levels in the supernatant (99.9±3.7 to 1010.1±96.1 pg/mL and 10.3±0.7 to 58.0±1.8 pg/mL, respectively) at 6 h and 48 h of the GCE treatment were shown to be significantly higher than those in the untreated controls (n=3, $P<0.05$) (Fig. 1E and 1F). As MMP1 expression was shown to be considerably higher than that of MMP9, I focused on MMP1 in further research.

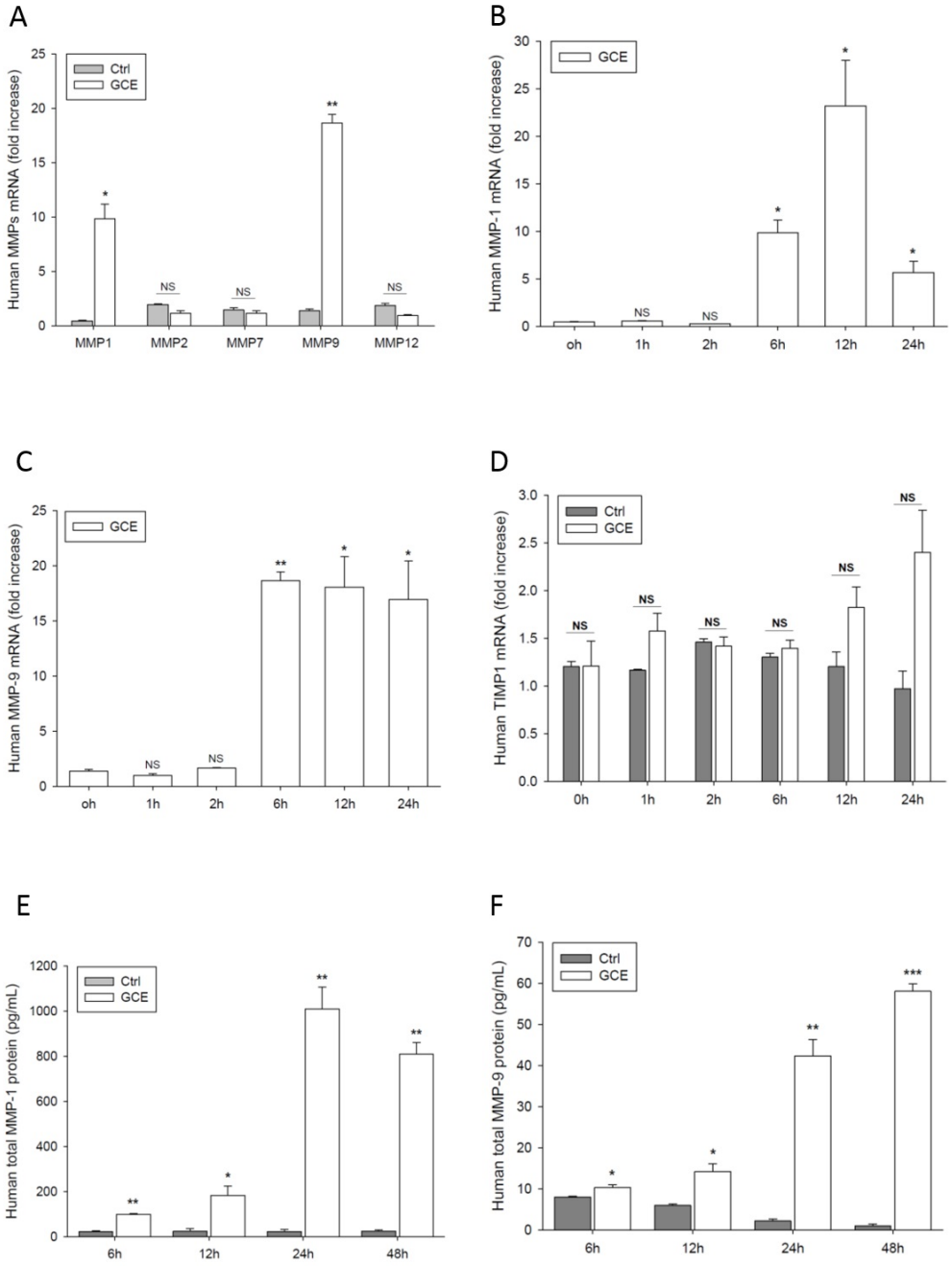


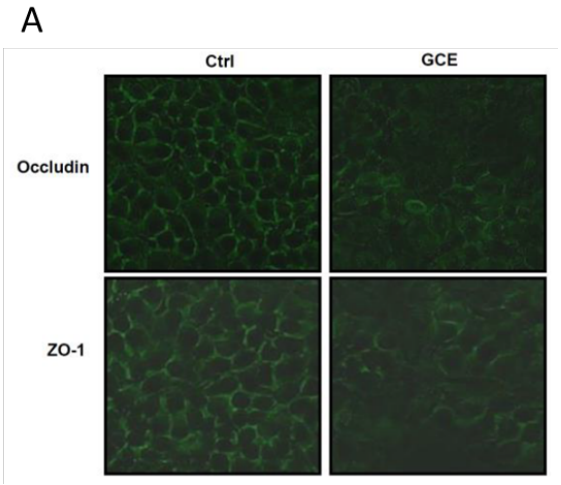
Figure 1. Expression of matrix metalloproteinases (MMPs) and TIMP in human

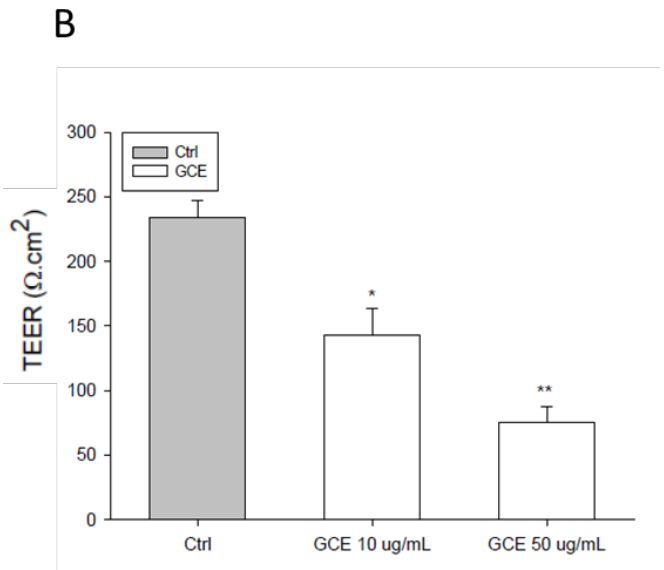
airway epithelial cells (H292) treated with German cockroach extract (GCE) for up to 48 h. (A) *MMP1, 2, 7, 9*, and *12* expression at one time point. Time-dependent expression of (B) *MMP1*, (C) *MMP9*, and (D) *TIMP1* was analyzed using real-time PCR. (E) MMP1 and (F) MMP9 levels were analyzed in the cell culture supernatants. Data are presented as mean ± SEM obtained in three independent experiments. **P*<0.05, ***P*<0.01, compared with the untreated or cells at 0 h; NS, not significant.

2. GCE treatment leads to the disruption of tight junctions between the airway epithelial cells

The cells were treated with GCE for 24 h, and stained with anti-occludin or anti-ZO1 antibody, since these proteins can indicate tight junction disruptions. Occludin plays crucial roles in the maintenance of tight junctions, signaling, and junction remodeling, while ZO1 is involved in the establishment of the belt-like tight junctions. Additionally, these proteins closely interact, although the nature and functional significance of these interactions are poorly understood[23]. As shown in Figure 2A, the untreated control samples showed strong immunofluorescent staining, with clear intercellular borders, while the GCE-stimulated cells displayed irregular and smeared staining patterns. Consistent with the tight junction disruption, GCE treatment significantly and dose-dependently decreased transepithelial electrical resistance (TEER) (Fig. 2B). However, the levels of both occludin and *ZO1* mRNA

did not significantly differ between the untreated control and GCE-treated cells (Fig. 2C and 2D), whereas the protein levels of occludin and ZO1 dose-dependently decreased following the GCE treatment (Fig. 2E and 2F).





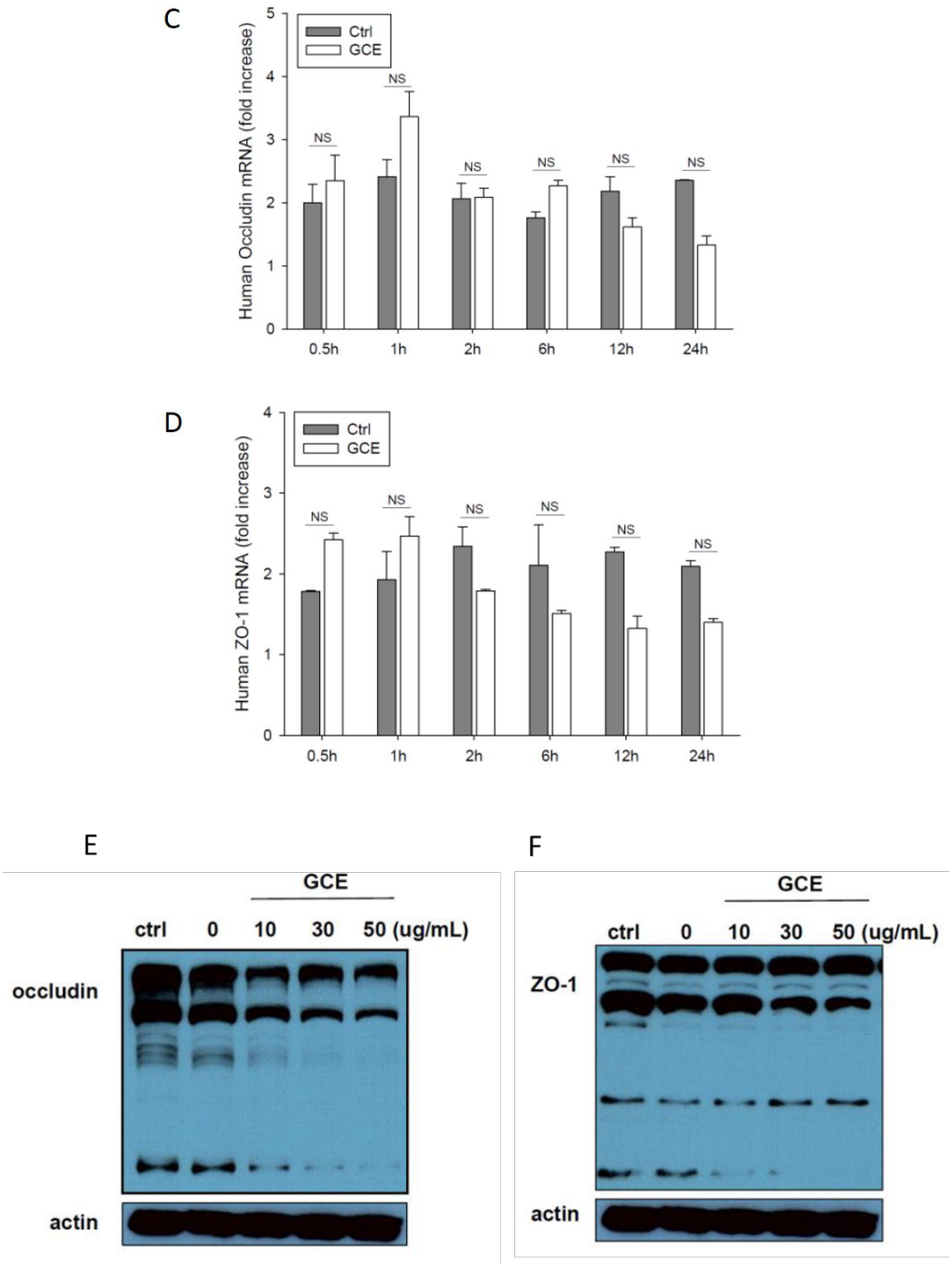


Figure 2. Effects of German cockroach extract (GCE) on tight junction proteins in

H292 cells. (A) Occludin and ZO1 localization on the GCE-stimulated cells was detected by immunofluorescence staining and observed under confocal microscopy. (B) Transepithelial electrical resistance following the GCE treatment for 24 h. (C) Occludin and (D) ZO1 mRNA expression. (E) Occludin and (F) ZO1 protein expression levels at the indicated time points. Data are presented as mean ± SEM obtained in three independent experiments. *P<0.05 and **P<0.01, compared with the untreated controls. Representative images are presented.

3. GCE-induced MMP1 expression leads to tight junction disruptions

To determine the relationship between tight junction disruptions and MMP1 release, the cells were pretreated with a MMP inhibitor, GM6001, that can inactivate MMP1, 2, 3, 7, 8, 9, 12, 14, and 26 for 1 h prior to the 24-h treatment with GCE. Confocal microscopy analysis showed that GCE-treated cells had discontinuous tight junctions, together with the altered localization of occludin and ZO1, whereas GM6001-pretreated cells had continuous tight junctions, similar to those in the untreated controls (Fig. 3A). Furthermore, we examined the effect of *MMP1* knockdown, showing that this leads to a considerable increase in tight junction protein levels (Fig. 3B and 3C).

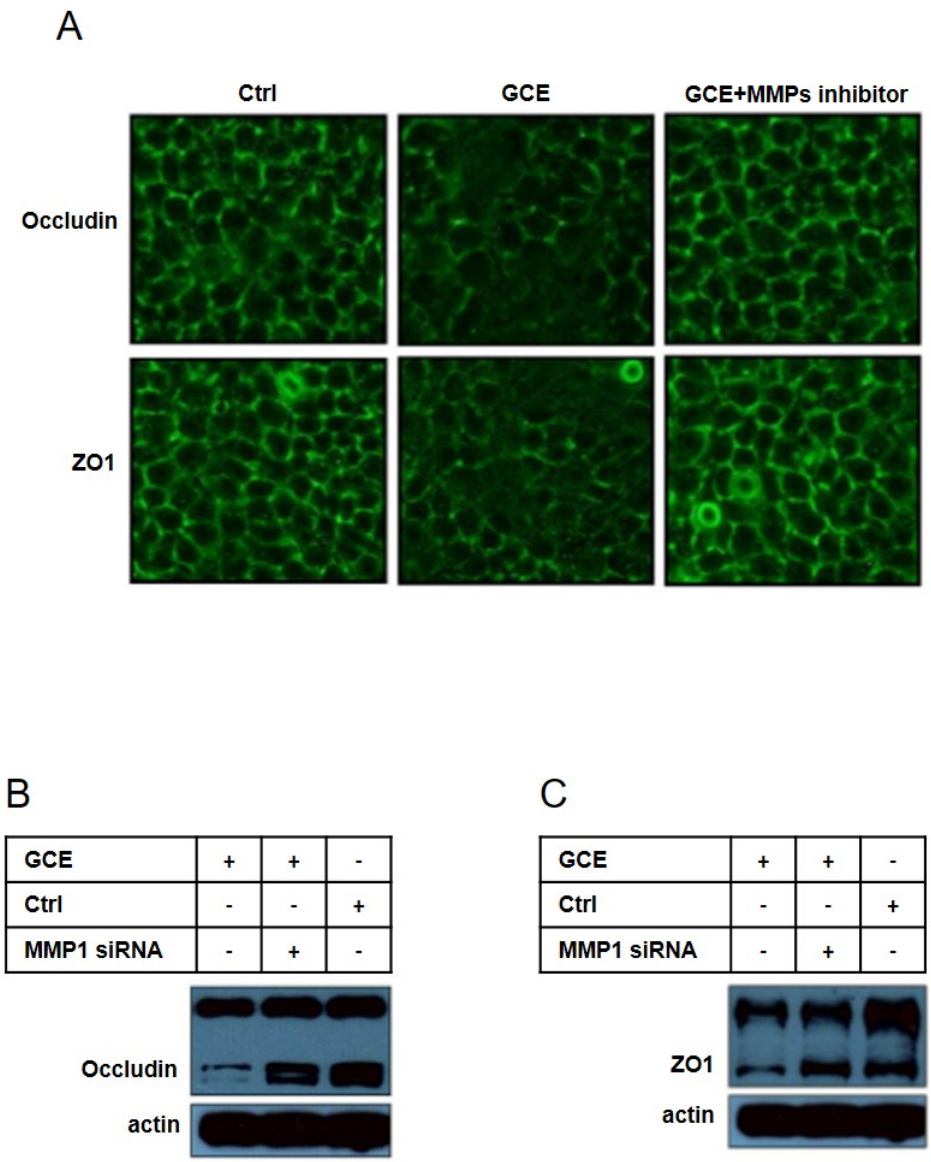


Figure 3. Effects of matrix metalloproteinase (MMP) expression inhibition on tight junction disruption in H292 cells. (A) Representative images showing occludin and

ZO1 localization in cells pretreated with MMP inhibitor GM6001 and German cockroach extract (GCE). (B) Occludin and (C) ZO1 expression levels in cells transiently transfected with MMP1 siRNA and treated with GCE. Representative images of three independent experiments are presented.

4. ETS1 and SP1 regulate GCE-induced MMP1 expression that leads to tight junction disruptions

Since the increase in MMP1 expression was shown to correlate with asthma severity, and this dysregulation is reflected in pulmonary architecture remodeling and inflammation[20], the mechanisms underlying the GCE-associated regulation of MMP1 expression were analyzed. Based on the previous reports, several transcriptional factors, including ETS1, SP1, AP1, and NF-κB, were focused as they are known to play important roles in MMP1 regulation[27, 28]. To evaluate their roles, the cells were treated with GCE (50 μg/mL), and observed their expression level changes with time. As shown in Figure 4A and 4B, respectively, ETS1 expression was strongly induced by GCE at 6 h and SP1 levels were the highest at 1 h after the initiation of the treatment (2.7-fold and 1.5-fold, respectively, $P<0.01$, compared with the untreated controls; n=3). However, no changes in the AP1 and NF-κB levels were observed (data not shown). Next, whether these transcription factors can regulate GCE-induced MMP1 expression were investigated, and showed that the siRNAs targeting ETS1,

ETS2, and SP1 inhibit the GCE-induced expression of MMP1 (74.4%, 75.2%, and 60.7% reduction, respectively, compared with the GCE-stimulated cells; n=3) (Fig. 4C and 4D). Furthermore, the cells transfected with siRNA against ETS1 or SP1 were treated with GCE for 24 h, and we assessed tight junction protein expression. While no effects on occludin protein levels were observed, the inhibition of ETS1 and SP1 expression prevented ZO1 protein loss in cells treated with GCE, compared with that in the controls (Fig. 4E and 4F).

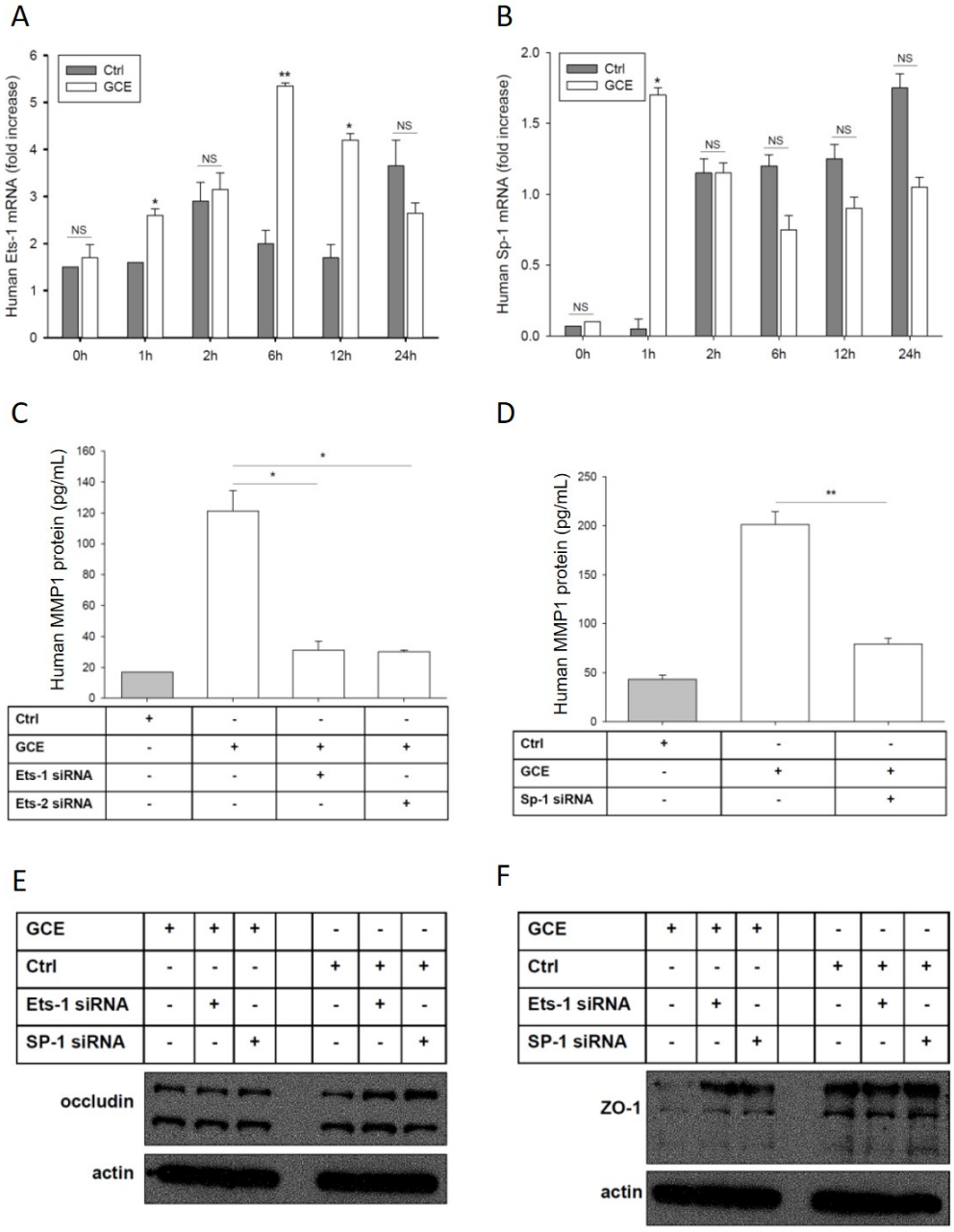


Figure 4. German cockroach extract (GCE)-induced regulation of tight junction

disruption in H292 cells. (A) ETS1 expression in GCE-treated cells. (B) SP1 expression in GCE-treated cells. (C and D) MMP1 expression following the transient transfection of cells with ETS1, ETS2, or SP1 siRNA and GCE treatment. Cell lysates were collected to assess the expression of (E) occludin and (F) ZO1 proteins. Data are represented as mean ± SEM obtained in three independent experiments; *P<0.05, **P<0.01, compared with the untreated control or GCE-stimulated cell cultures. Images are representative of three individual experiments.

5. ERK through TLR2 controls GCE-induced MMP1 expression, leading to tight junction protein level decrease

Furthermore, to elucidate the mechanism underlying the observed effects, GCE-induced MAPK pathway activation was investigated using specific antibodies against phosphorylated ERK1/2 (Fig. 5A), and phosphorylated p38 and phosphorylated JNK (data not shown). Cell treatment with PD98059, a potent inhibitor of MAPK/ERK, significantly decreased GCE-induced MMP1 expression (n=3; 65.9% reduction, compared with that in the GCE only-treated cells; P<0.05; Fig. 5B). While the treatment with PD98059 inhibitor did not affect GCE-induced occludin decrease, it did prevent the GCE-induced decrease in ZO1 levels (Fig. 5C and 5D). Additionally, it was investigated whether TLRs, which play a key role in the innate immune system responses, can affect GCE-MMP1 release, leading to tight

junction alterations. A specific anti-TLR2 antibody prevented the GCE-mediated increase in MMP1 expression (97% of reduction, compared with that in the GCE only-treated cells; n=3; $P<0.01$; Fig. 6A). The inhibition of GCE-induced decrease in occludin and ZO1 protein levels was observed as well (Fig. 6B and 6C).

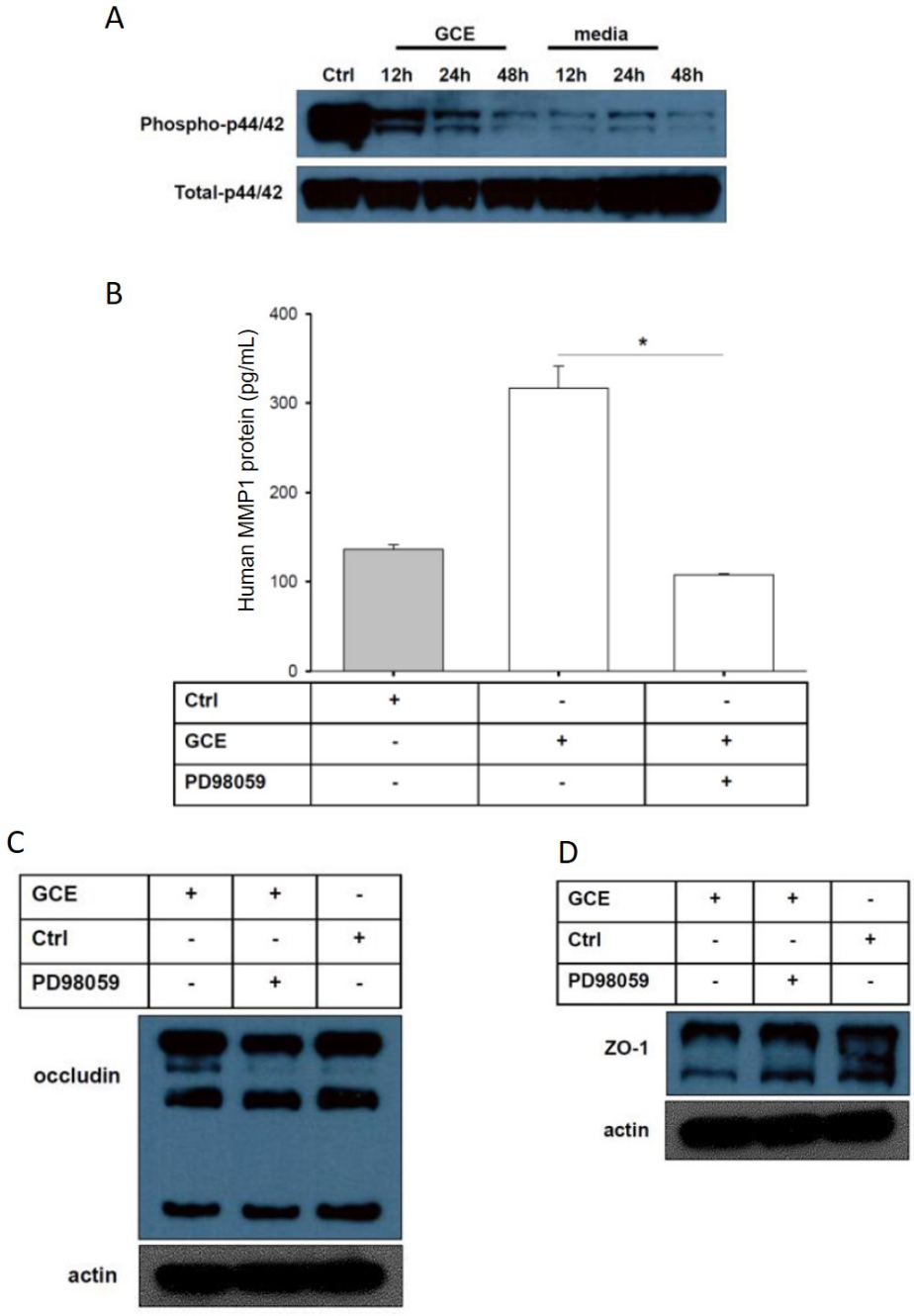


Figure 5. Analysis of the regulatory mechanisms affecting matrix metalloproteinase

(MMP)1 expression. (A) Phosphorylated and total ERK1/2 expression levels in H292 cells pretreated with PD98059 for 1 h before German cockroach extract (GCE) treatment for 24 h. (B) MMP1 expression in the treated cell supernatant. (C and D) The expression of occludin and ZO1 proteins in the untreated cells and those treated with the inhibitor. Data are presented as mean ± SEM obtained in three independent experiments. *P<0.05, compared with the GCE only-stimulated cells. Images are representative of the results obtained in three independent experiments.

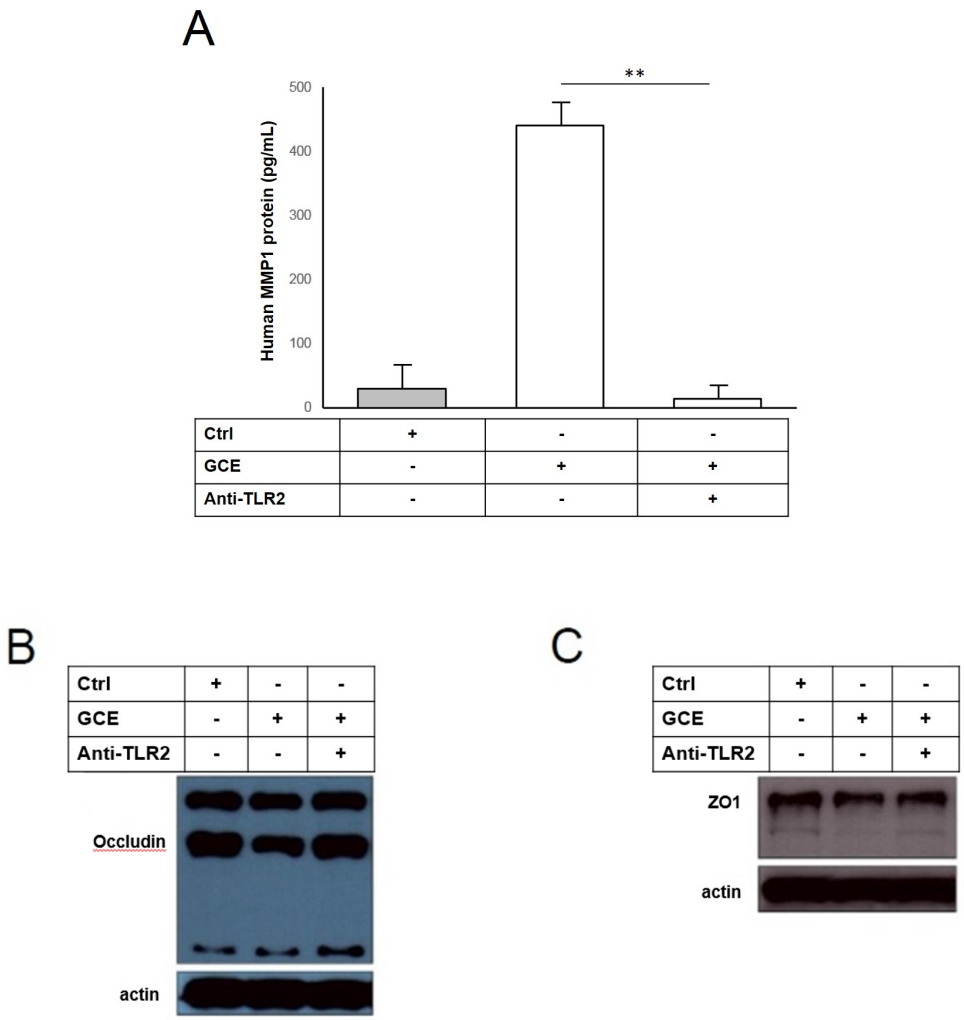


Figure 6. Toll-like receptor involvement in tight junction regulation. (A) Matrix metalloproteinase (MMP)1 expression in H292 cells were treated with anti-TLR2 antibody and German cockroach extract (GCE). (B and C) Occludin and ZO1 protein expression in the treated and untreated cells. Data are presented as mean ± SEM

obtained in three independent experiments. **P<0.01, compared with the GCE only-treated cells. Images are representative of the results obtained in three independent experiments.

IV. DISCUSSION

Cockroach allergen was shown to contain a protease that associates with PAR2 and TLR2 motif, which can modulate inflammatory cytokine and chemokine activity, leading to the direct activation of innate immune system[5,8]. Furthermore, cockroach extract increases bronchial airway epithelial permeability, but the roles of different components have not been elucidated[29]. Attempts at isolation and characterization of the serine protease are ongoing, and have led to the enrichment of the active protease, virtually devoid of endotoxin[8]. Here, this study demonstrated *in vitro* that GCE significantly induces MMP1 expression. MMPs are key molecules involved in cell proliferation and migration, tissue growth, remodeling, and regeneration, which have high proteolytic activity and broad substrate specificity[12]. MMPs constitute a large family of Zn-dependent endoproteinases. So far, at least 25 distinct MMP family members have been identified and, based on their molecular structure, substrate specificity and mechanism of activation, classified into four groups; archetypal MMPs, matrilysins, gelatinases, and furin-activated MMPs. Recently, MMPs were identified

as likely to be involved in the pathogenesis of asthma, primarily in the asthma-associated airway remodeling[31]. Although the role of MMP9 in asthma pathogenesis has been extensively studied, the levels of other MMPs, including MMP1, 2, 3, or 12 in individuals with asthma were shown to be increased as well, indicating that their levels in sputum, BAL, and exhaled breath condensates (EBC) correlate with disease exacerbation[32,33]. Additionally, it was reported that MMP1 is highly expressed in airway epithelial cells, inflammatory cells and even airway smooth muscle cells in the asthmatic patients[27,34,35].

For many years, it has been described that the airway epithelium plays a key role in the development of asthma, since it is the first site of contact with environmental substances and expresses several of the recently identified asthma susceptibility genes[31]. Tight junctions are located at the most apical side of the cell layer, forming the closest site of contact between neighboring cells and regulating the macromolecular and ionic permeability and polarity of the epithelial barrier. Tight junction complexes are formed by transmembrane spanning proteins, including claudins, occludin and junctional adhesion molecule, which link neighbouring cells. On the intracellular side, these transmembrane proteins are connected to the actin cytoskeleton via protein complexes including ZO proteins. In addition, bronchial epithelial cells are able to influence the immunological barrier through their ability to release immunostimulatory and immunomodulatory mediators such as cytokines, chemokines and growth factors. In asthma, there is evidence that the epithelial barrier properties are impaired, which may be caused by intrinsic factors, e.g. genetic

polymorphisms, or by environmental factors such as respiratory viruses, cigarette smoke, pollution and allergens[36,37]. However, only a small number of studies investigated cockroach allergen effects on the epithelial barrier[5]. In this study, occludin and ZO1 were selected as the indicators of tight junction disruption, because occludin is key component of a tight seal formed between the cells, while ZO1 directly interacts with occludin, to ensure proper localization of the tight junctions[23]. This study showed that GCE induces morphological changes and the dose-dependent reduction of tight junction protein levels, in combination with an increase in the epithelial permeability, regardless of occludin and *ZO1* gene expression levels, suggesting that GCE may lead to the degradation of epithelial tight junction proteins. Previously, cockroach antigen treatment was shown to induce VEGF expression and decrease electrical resistance in the bronchial epithelial cells, although the cockroach role was not investigated[30]. To date, the activity of cockroach allergen has been explained by its effects on PAR2, which can lead to disease pathogenesis or exacerbation[5]. It was previously suggested that these cockroach allergen activities mediated by PAR2 may be further complicated by the involvement of TLRs, showing that cockroach allergen contains both TLR4 and TLR2 motives[7], and that PAR2 and TLR4 signal cooperatively[38,39].

These data demonstrate that GCE induced MMP1 expression and tight junction disruption, which play important roles in disease-associated airway remodeling[26]. Therefore, it was investigated whether MMP1 cleaves occludin and ZO1, the components of tight junctions. The results show that GM6001, a potent MMP

inhibitor, and *MMP1* siRNA prevent the degradation of tight junction proteins, showing that tight junction disruption may be increased by GCE-induced MMP1 expression in airway epithelial cells. MMP1 is a collagenase that can be detected in the small airways and lung parenchyma of asthmatic patients[40,41]. MMP1 expression was shown to directly correlate with the airway obstruction in BAL fluid, and that is associated with the development of asthma symptoms although the mechanisms remained unclear[27,35,42]. Inadequate tight junction formation has been confirmed in asthmatic samples compared with the normal cultures, using anti-ZO1 and anti-occludin antibodies, suggesting that respiratory viruses, air pollutants, and proteolytically active allergens can cause the epithelial desquamation through tight junction disruption and the disruption of epithelial barrier function[25,27,37,43].

Although the role of MMPs in asthma pathogenesis has been extensively researched, their inhibitors for potential therapeutic purposes have rarely been developed. Therefore, understanding the molecular mechanisms underlying the regulation of MMP1 expression may help identify potential targets for the prevention of disease development. MMP1 expression is tightly regulated at the levels of transcription, post-transcription, and post-translation[44]. At the transcriptional level, the promoter of *MMP1,* which contains the adjacent binding sites for ETS1 and AP1 transcription factors, is known to be crucial for the regulation of *MMP1* expression[28,45]. The distal 1.5-kb segment of the *MMP1* promoter was identified as necessary for the direct induction of *MMP1* expression by cigarette smoke[46]. Moreover, NF-κB is a main specific transcription factor that localizes to the promoter

regions of MMPs in response to various stimuli[28,29]. Here, the involvement of these transcription factors, ETS1, AP1, SP1, and NF-κB, in the GCE-induced MMP1 expression was analyzed, showing that both ETS1 and SP1 are significantly activated and that their inhibition attenuates MMP1 release and ZO1 degradation. The signaling molecules mediating GCE-induced MMP1 release was investigated, and the TLR2 and ERK/MAPK pathway activation was shown to be required for the increase in MMP1 levels. ERK/MAPK signaling molecules were shown to regulate ETS1 and SP1 translocation induced by GCE, following the TLR2 activation. MMP1 inhibition prevented tight junction degradation. Although TLRs are expressed on the majority of cells, they alone are not sufficient for the recognition of pathogens without coreceptors and adaptor molecules[8]. The role of TLR2 in asthma is controversial, since different studies showed that it can act as an enhancer or inhibitor of the allergy-related airway inflammation during sensitization[47,48]. German cockroach frass was suggested to contain a TLR2 agonist that directly induces neutrophils infiltration and further exacerbation of airway inflammation[8]. Furthermore, it was reported that *Staphylococcus aureus* induces MMP1 expression in human bronchial epithelial cells through the activation of TLR2/EGF receptor signaling axis[48]. The AP1, NF-κB, SP1, and ETS transcription factors and MAPK family members were shown to play crucial roles in the MMP induction. MMP1 expression induced by one of the extracellular matrix proteins, tenascin-C, was shown to depend on ERK1/2, JNK, and p38 MAPK activation in the airway smooth muscle cells[27]. Cockroach frass-induced MMP9 expression can be upregulated by ERK activation and AP1 nuclear translocation[49].

V. CONCLUSION

The diversity of cockroach allergens induce a multifaceted immune response involving both the innate and adaptive pathways of immune system, which are activated by enzymatic protease activity.

This study suggested that protease activity in GCE play an important role modulating airway epithelial cell layers with the inflammatory stimulus. It demonstrated here that GCE induces an increase in the MMPs release, especially MMP1, which is known to be pro-remodeling factor. MMP1 plays a crucial role in the tight junction disruption in airway epithelial cells. ZO1 and occludin protein expression levels in airway epithelial cells by GCE stimulation was influenced by MMP1 inhibitor, so that this study demonstrated that the influence of MMP1 to airway epithelial cells disruption. In order to assist the future development of MMP1 inhibitor, the transcription factors were identified, such as ETS1 and SP1, and signaling molecules, such as TLR2 and ERK/MAPK, regulating MMP1 expression. These molecules may represent potential targets for the development of novel treatments for asthma.

REFERENCES

1. Lombardi C, Savi E, Ridolo E, Passalacqua G, Canonica GW. Is allergic sensitization relevant in severe asthma? Which allergens may be culprit? World Allergy Organ J 2017;10:2.

2. Sohn MH, Kim KE. The cockroach and allergic diseases. Allergy Asthma Immunol Res 2012;4:264-9.

3. O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, et al. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. N Engl J Med 1991;324:359-63.

4. Rosenstreich DL, Eggleston P, Kattan M, Baker D, Slavin RG, Gergen P, et al. The role of cockroach allergy and exposure to cockroach allergen in causing morbidity among inner-city children with asthma. N Engl J Med 1997;336:1356-63.

5. Page K. Role of cockroach proteases in allergic disease. Curr Allergy Asthma Rep 2012;12:448-55.

6. Yong TS, Lee JS, Lee J, Park SJ, Jeon SH, Ree HI, et al. Identification and purification of IgE-reactive proteins in German cockroach extract. Yonsei Med J 1999;40:283-9.

7. Jeong KY, Kim C, Yong TS. Enzymatic activities of allergen extracts from three species of dust mites and cockroaches commonly found in Korean home. Korean J

Parasitol 2010;48:151–5

8. Page K, Lierl KM, Hughes VS, Zhou P, Ledford JR, Wills-Karp M. TLR2-mediated activation of neutrophils in response to German cockroach frass. J Immunol. 2008;180(9):6317-24.

9. Hughes VS, Page K. German cockroach frass proteases cleave pro-matrix metalloproteinase-9. Exp Lung Res 2007;33:135-50.

10. Kelly EA, Jarjour NN. Role of matrix metalloproteinases in asthma. Curr Opin Pulm Med 2003;9:28-33.

11. Vandenbroucke RE, Dejonckheere E, Libert C. A therapeutic role for matrix metalloproteinase inhibitors in lung diseases? Eur Respir J 2011;38:1200-14.

12. Grzela K, Strzelak A, Zagorska W, Grzela T. Asthma - From Childhood Asthma to ACOS Phenotypes: InTech; 2016. p.41-69.

13. Hendrix AY, Kheradmand F. The Role of Matrix Metalloproteinases in Development, Repair, and Destruction of the Lungs. Prog Mol Biol Transl Sci 2017;148:1-29

14. Fingleton B. MMPs as therapeutic targets--still a viable option? Semin Cell Dev Biol 2008;19:61-8.

15. Suzuki R, Kato T, Miyazaki Y, Iwata M, Noda Y, Takagi K, et al. Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases in sputum

from patients with bronchial asthma. J Asthma 2001;38:477-84.

16. .Royce SG, Shen M, Patel KP, Huuskes BM, Ricardo SD, Samuel CS. Mesenchymal stem cells and serelaxin synergistically abrogate established airway fibrosis in an experimental model of chronic allergic airways disease. Stem Cell Res 2015;15:495-505.

17. Holgate ST. A brief history of asthma and its mechanisms to modern concepts of disease pathogenesis. Allergy Asthma Immunol Res 2010;2:165-71.

18. Corry DB, Rishi K, Kanellis J, Kiss A, Song LZ, Xu J, et al. Decreased allergic lung inflammatory cell egression and increased susceptibility to asphyxiation in MMP2-deficiency. Nat Immunol 2002;3(4):347–53.

19. Corry DB, Kiss A, Song LZ, Song L, Xu J, Lee SH, et al. Overlapping and independent contributions of MMP2 and MMP9 to lung allergic inflammatory cell egression through decreased CC chemokines. FASEB J. 2004;18:995–7.

20. Rogers NK, Clements D, Dongre A, Harrison TW, Shaw D, Johnson SR. Extra-cellular matrix proteins induce matrix metalloproteinase-1 (MMP-1) activity and increase airway smooth muscle contraction in asthma. PLoS One 2014;9:e90565.

21. Loxham M, Davies DE, Blume C. Epithelial function and dysfunction in asthma. Clin Exp Allergy 2014;44:1299-313.

22. Naveed SU, Clements D, Jackson DJ, Philp C, Billington CK, Soomro I, et al.

Matrix Metalloproteinase-1 Activation Contributes to Airway Smooth Muscle Growth and Asthma Severity. Am J Respir Crit Care Med 2017;195: 1000-9.

23. Shin K, Fogg VC, Margolis B. Tight junctions and cell polarity. Annu Rev Cell Dev Biol 2006;22:207-35.

24. Holgate ST. Epithelium dysfunction in asthma. J Allergy Clin Immunol 2007;120:1233-44; quiz 45-6.

25. Holgate ST. The airway epithelium is central to the pathogenesis of asthma. Allergol Int 2008;57:1-10.

26. Vermeer PD, Denker J, Estin M, Moninger TO, Keshavjee S, Karp P, et al. MMP9 modulates tight junction integrity and cell viability in human airway epithelia. Am J Physiol Lung Cell Mol Physiol 2009;296:L751-62.

27. Yan C, Boyd DD. Regulation of matrix metalloproteinase gene expression. J Cell Physiol 2007;211:19-26.

28. Hadler-Olsen E, Fadnes B, Sylte I, Uhlin-Hansen L, Winberg JO. Regulation of matrix metalloproteinase activity in health and disease. FEBS J 2011;278:28-45.

29. Antony AB, Tepper RS, Mohammed KA. Cockroach extract antigen increases bronchial airway epithelial permeability. J Allergy Clin Immunol 2002;110:589-95.

30. Grzela K, Litwiniuk M, Zagorska W, Grzela T. Airway Remodeling in Chronic

Obstructive Pulmonary Disease and Asthma: the Role of Matrix Metalloproteinase-9. Arch Immunol Ther Exp (Warsz) 2016;64:47-55.

31. Erlewyn-Lajeunesse MD, Hunt LP, Pohunek P, Dobson SJ, Kochhar P, Warner JA, et al. Bronchoalveolar lavage MMP-9 and TIMP-1 in preschool wheezers and their relationship to persistent wheeze. Pediatr Res 2008;64:194-9.

32. Karakoc GB, Yukselen A, Yilmaz M, Altintas DU, Kendirli SG. Exhaled breath condensate MMP-9 level and its relationship wıth asthma severity and interleukin-4/10 levels in children. Ann Allergy Asthma Immunol 2012;108:300-4.

33. Rajah R, Nachajon RV, Collins MH, Hakonarson H, Grunstein MM, Cohen P. Elevated levels of the IGF-binding protein protease MMP-1 in asthmatic airway smooth muscle. Am J Respir Cell Mol Biol 1999;20:199-208.

34. Dolhnikoff M, da Silva LF, de Araujo BB, Gomes HA, Fernezlian S, Mulder A, et al. The outer wall of small airways is a major site of remodeling in fatal asthma. J Allergy Clin Immunol 2009;123:1090-7.

35. Wan H, Winton HL, Soeller C, Gruenert DC, Thompson PJ, Cannell MB, et al. Quantitative structural and biochemical analyses of tight junction dynamics following exposure of epithelial cells to house dust mite allergen Der p 1. Clin Exp Allergy 2000;30:685-98.

36. Blume C, Swindle EJ, Dennison P, Jayasekera NP, Dudley S, Monk P, et al.

Barrier responses of human bronchial epithelial cells to grass pollen exposure. Eur Respir J 2013;42:87-97.

37. Nhu QM, Shirey K, Teijaro JR, Farber DL, Netzel-Arnett S, Antalis TM, et al. Novel signaling interactions between proteinase-activated receptor 2 and Toll-like receptors in vitro and in vivo. Mucosal Immunol 2010;3:29-39.

38. Rallabhandi P, Nhu QM, Toshchakov VY, Piao W, Medvedev AE, Hollenberg MD, et al. Analysis of proteinase-activated receptor 2 and TLR4 signal transduction: a novel paradigm for receptor cooperativity. J Biol Chem 2008;283:24314-25.

39. Segura-Valdez L, Pardo A, Gaxiola M, Uhal BD, Becerril C, Selman M. Upregulation of gelatinases A and B, collagenases 1 and 2, and increased parenchymal cell death in COPD. Chest 2000;117:684-94.

40. Imai K, Dalal SS, Chen ES, Downey R, Schulman LL, Ginsburg M, et al. Human collagenase (matrix metalloproteinase-1) expression in the lungs of patients with emphysema. Am J Respir Crit Care Med 2001;163:786-91.

41. Cataldo DD, Gueders M, Munaut C, Rocks N, Bartsch P, Foidart JM, et al. Matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases mRNA transcripts in the bronchial secretions of asthmatics. Lab Invest 2004;84:418-24.

42. Xiao C, Puddicombe SM, Field S, Haywood J, Broughton-Head V, Puxeddu I, et al. Defective epithelial barrier function in asthma. J Allergy Clin Immunol 2011;128:549-56.e1-12.

43. Rutter JL, Mitchell TI, Butticè G, Meyers J, Gusella JF, Ozelius LJ, et al. A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription. Cancer Res 1998;58:5321-5.

44. Liu T, Wang P, Cong M, Zhang D, Liu L, Li H, et al. Matrix metalloproteinase-1 induction by diethyldithiocarbamate is regulated via Akt and ERK/miR222/ETS-1 pathways in hepatic stellate cells. Biosci Rep 2016;36(4).

45. Wallace AM, Mercer BA, He J, Foronjy RF, Accili D, Sandford AJ, et al. Functional characterization of the matrix metalloproteinase-1 cigarette smoke-responsive region and association with the lung health study. Respir Res 2012;13:79.

46. Redecke V, Häcker H, Datta SK, Fermin A, Pitha PM, Broide DH, et al. Cutting edge: activation of Toll-like receptor 2 induces a Th2 immune response and promotes experimental asthma. J Immunol 2004;172:2739-43.

47. Patel M, Xu D, Kewin P, Choo-Kang B, McSharry C, Thomson NC, et al. TLR2 agonist ameliorates established allergic airway inflammation by promoting Th1 response and not via regulatory T cells. J Immunol 2005;174:7558-63.

48. Homma T, Kato A, Sakashita M, Norton JE, Suh LA, Carter RG, et al. Involvement of Toll-like receptor 2 and epidermal growth factor receptor signaling in epithelial expression of airway remodeling factors. Am J Respir Cell Mol Biol 2015;52:471-81.

49. Page K, Hughes VS, Bennett GW, Wong HR. German cockroach proteases regulate matrix metalloproteinase-9 in human bronchial epithelial cells. Allergy 2006;61:988-95.

ABSTRACT (IN KOREAN)

독일바퀴항원에 의해 유도된 matrix metalloproteinase 발현이 인체기도상피세포 치밀연접에 미치는 영향

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지 혜 미

독일바퀴항원은 기도상피세포의 기능 저하를 유도하여 천식의 발생에 영향을 미치는 중요한 알레르겐이다. 최근 기도상피세포의 치밀연접이 기도상피세포의 기능을 유지하는데 핵심적인 역할을 하는 것으로 보고되었는데, 독일바퀴항원에 노출 된 기도상피세포의 치밀연접의 손상이 천식에 의한 기도변화를 유도하는 것으로 생각된다. Matrix metalloproteinase (MMP)는 세포외질의 구성성분 분해에 관여하는 단백분해효소로써, 천식환자의 기도변화에 영향을 미치는 것으로 추측되나 그 기전은 잘 알려져 있지 않다.

이에 본 연구에서는 MMP1이 독일바퀴항원에 의해 유도되는 것을 확인하고, 독일바퀴 항원에 의해 유도된 MMP1이 인체기도상피세포의 치밀연접에 미치는 영향을 연구하고자 하였다.

인체기도상피세포를 독일바퀴항원으로 처리한 후 mRNA, 실시간

중합효소연쇄반응, 효소면역법 등을 이용하여 MMP의 발현 정도를 측정하고 MMP가 독일바퀴항원에 의해 유도되는 것을 증명하기 위해 항원처리 전 MMP 억제제인 GM6001을 처리하여 변화를 확인하였다. 독일바퀴항원에 의해 유도된 MMP가 치밀연접에 미치는 영향을 확인하기 위해 면역형광염색과 상피관통 전기저항 측정을 시행하였다. 발현의 기전을 확인하기 위해 MAPK/ERK 활성효소 억제제인 PD98059를 처리하여 변화를 측정하였고, ETS1, SP1에 대한 siRNA와 anti-TLR2 항체를 처리하여 MMP 발현의 신호전달경로를 조사하였다.

독일바퀴항원으로 기도상피세포를 처리한 후 MMP의 발현이 증가하였는데, 특히 MMP1이 의미 있게 증가하였다. 독일바퀴항원으로 기도상피세포를 처리한 후 면역형광염색을 확인하였을 때 기도상피세포의 면역형광염색 정도가 감소하고 상피관통 전기저항이 독일바퀴항원 농도가 높을수록 낮아짐을 확인함으로써 기도상피세포의 치밀연접부위 단백질이 독일바퀴항원 처리에 의해 손상 받음을 알 수 있었다. 반면 MMP1을 억제한 경우 기도상피세포의 면역형광염색이 회복되는 것을 확인함으로써 MMP1이 독일바퀴항원 처리에 의한 기도상피세포의 치밀연접 손상에 관여함을 확인하였다. ETS1, SP1의 발현을 억제시킨 경우 MMP1의 증가가 감소하고, 치밀연접부위 단백질의 발현이 감소하는 것으로 보아 독일바퀴항원에 의해 유도되는 MMP1은 ETS1, SP1 전사인자에 의해 조절됨을 확인할 수 있었고 anti-TLR2 항체에 의해서도 MMP1의 증가감소, 치밀연접부위 단백질의 발현이 감소하는 것으로 보아 TLR2를 통한 신호전달체계를 통하여 독일바퀴항원에 의한 MMP1

발현이 조절되는 것을 확인하였다.

결론적으로 본 연구에서는 독일바퀴항원에 의해 유도된 MMP1이 인체기도상피세포의 치밀연접 단백질의 손상을 유도함을 확인하였으며 이는 TLR2를 통한 MAPK/ERK 활성경로를 거쳐 발생됨을 알 수 있었다. 이러한 결과를 바탕으로 향후 기도상피세포 손상과 관련된 질환의 치료제 개발에 도움이 될 것으로 기대한다.

핵심되는 말 : 독일바퀴항원, matrix metalloproteinase, 치밀연접, 기도상피세포, 천식