Role of MAP kinases in chitinase induced IL-8 secretion in human airway epithelial cells

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Role of MAP kinases in chitinase induced IL-8 secretion in human airway epithelial cells

Directed by Professor Kyu-Earn Kim

The Master's Thesis submitted to the Department of Medical Science, the Graduate School of Yonsei University in partial fulfillment of the requirements for the degree of Master of Medical Science

Jung Yeon Hong

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This certifies that the Master's thesis

of Jung Yeon Hong is approved.

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The Graduate school

Yonsei University

December 2007

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늘 바쁘고 상냥하지도 못했던 외손녀, 따뜻한 식사와 걱정으로 6년째 돌봐주신 외할머니, 자주 찾아뵙지 못한 친할머니, 건강하시고 앞으로는 착한 손녀가 되겠습니다. 멀리서 항상 딸들 걱정이셨던 부모님, 씩씩하게 살아가고, 착하게 살아가는 것과 효도란 것이 무엇인지 아버지, 어머니로부터 배웠습니다. 항상 존경하고, 사랑합니다. 무엇보다도 앞으로 항상 건강

하시길 바랍니다. 그리고 공부한다는 명목하에 불효를 많이 범했는데, 이제 장녀로서 부모님께 앞으로는 기쁜 소식과 즐거움만 드리겠다고 스스로 다짐해 봅니다. 그리고 항상 언니를 찾던 나의 동생 지예, 이제 대학생이 된 네가 언니는 자랑스럽고 대견하단다. 다른 나라에서 공부하지만 항상 건강하고 후회 없는 4년이 되길 빌며, 멀리서도 항상 응원해주어 너무 고마워.

어머니, 아버지의 기도에 응답하기 위해 이제는 성당을 열심히 나가야 할 것 같습니다. 늘 기회와 용기를 주시는 나의 하나님께 감사드립니다.

2008년 2월
홍정연

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ABSTRACT

Role of MAP kinases in chitinase induced IL-8 secretion in human airway epithelial cells

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(Directed by Professor Kyu-Earn Kim)

Chitinase is produced by a number of different chitin-coating organisms. Several studies have suggested that acidic mammalian chitinase (AMCase) is induced Th2-like airway inflammation. However, little is known on the immune response of exogenous chitinase frequently exposed daily life. IL-8 has been suggested to have a role in the pathogenesis of the allergenic inflammation of bronchial asthma. We examined whether *Streptomyces griseus* (*S. griseus*) chitinase induced IL-8 effect on airway epithelium and identified the relation between protein kinases and IL-8 production.

Cells were treated with *S. griseus* chitinase in different concentrations and times. IL-8 levels were determined by specific human IL-8

enzyme-linked immunosorbent assay (ELISA) and reverse transcriptase polymerase chain reaction (RT-PCR). And using a series of pharmacological inhibitors, we examined the upstream signalling pathway responsible for IL-8 expression in response to *S. griseus* chitinase.

In H292 cells, treatment with *S. griseus* chitinase increased IL-8 protein production and mRNA expression level depending on time and dose. Treatment with U0126 and SB202190, which blocks the activation of extracellular signal-regulated kinase (ERK) 1/2 and p38 mitogen activated protein kinase (MAPK), inhibited IL-8 expression significantly. However c-Jun NH2-terminal kinase (JNK) inhibitor had no effect. And we demonstrated that activation of Protein Kinase C (PKC) isoform ɣ was required of chitinase induced IL-8 expression by treatment of calphostin C and Ro-31-8220. In addition, through radicicol treatment, it was proven that Ras-Raf signalling regulated chitinase-induced IL-8 expression.

We concluded that *S. griseus* chitinase-induced IL-8 expression was regulated by activation of PKC, Ras-Raf, ERK and p38 MAPK in human airway epithelial cells.

Key words: chitinase, airway epithelial cell, IL-8, ERK, p38, PKC

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Ⅰ. INTRODUCTION

Chitinase is a chitin hydrolase which is used for defensing against infection with chitin coating organisms (innate immunity response), controling growth, molting and invading host in the life cycle of chitin containing organisms.[1,2,3] Recently, one study identified that acidic mammalian chitinase (AMCase) is potently induced via a T helper-2 (Th 2) specific Interleukin-13 (IL-13) -mediated pathway. And AMCase neutralization ameliorated Th 2 inflammation and airway hyperresponsiveness by inhibiting IL-13 pathway activation and chemokine induction. AMCase may thus be an important mediator of IL-13 induced response in Th 2-dominated disorder such as asthma.[2,3,4]

However, little is known on the immune response of exogenous chitinase from fungi, bacteria or parasites, frequently exposed daily life. Environmental factors that may alter airway reactivity, including allergens, cigarette smoke, air pollutants, viruses, fungi, bacteria and parasites,[5,6,7,8,9] have been demonstrated to increase IL-8 expression of airway epithelial cell. IL-8 is a member of the CXC chemokine family that has been suggested to play a pivotal role as a most important mediator in airway inflammatory disease.[10] Increased level of IL-8 has been found in the bronchoalveolar lavage fluid (BAL) of airway inflammation disease patients, which demonstrated the role of IL-8 in airway inflammation and innate immunity.[11] The mechanisms of pathophysiologial responses and stimuli of IL-8 were well identified. However, nothing is known about the role of exogenous chitinase induced-IL-8 expression.

To date, accumulating evidence of intracellular signal pathway indicates an important function of mitogen activated protein kinase (MAPK) in bronchial epithelial cells. MAPKs, serine/threonine protein kinases that are activated in response to various external stimuli, mediate important regulatory signals in the cell. Three major groups of MAPKs have been characterized in mammals: the p38, the extracellular signal-regulated kinase (ERK), and the c-Jun NH2-terminal kinase (JNK) MAPK.[12,13] MAPKs have been demonstrated to play an important role in the release and gene expression of IL-8 in response to the stimulation by *Mycoplasma pneumoniae*,[9] German cockroach extract[10] and respiratory syncytial virus (RSV)[8] in bronchial epithelial

cells.

The participation of protein kinase C (PKC) in MAPKs cascade activation after stimulation has been reported in various cell types. PKC is a serine/threonine protein kinase and well known important signalling intermediates in chronic airway diseases like asthma and chronic obstructive pulmonary disease (COPD).[14] PKC consists of at least 11 isoforms. Activation of the classical isoforms(α, βⅠ,βⅡ and γ) is dependent on Ca^{2+} and diacylglycerol (DAG). The novel PKCs isoforms(δ, ε, η μ and Θ) lack the Ca^{2+} binding domain and are activated by DAG not Ca^{2+}.[15,16]

We hypothesized that exogenous chitinase of *Streptomyce griseus*[1,17,18] (*S. griseus*) might also contribute to the airway inflammation. Thus, in this study we evaluate whether *S. griseus* chitinase induces IL-8 protein production and investigate intracellular signalling pathway which regulates chitinase-induced IL-8 expression in human airway epithelial cells.

Ⅱ. MATERIALS AND METHODS

1. Cell culture

Human epithelial lung carcinoma cell line, H292, (ATCC, MD, USA) was grown in RPMI 1640 (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin and streptomycin (GibcoBRL, NY, USA). Cells were maintained in humidified incubator at 37℃ with 5% CO_2. The cells were replenished with fresh media every 2-3 days. For each experiment, cells (3×10^5 cells/mL) were plated in 6-well culture plate.

2. *S. griseus* chitinase and reagents

Chitinase from *S. griseus* were purchased from Sigma (St. Louis, MO, USA). Chitinase was suspended by 1×PBS buffer (Hyclone, Logan, UT, USA). BAPTA/AM (a intracellular Ca^{2+} chelator),[20] Ro-31-8220 (a pan inhibitor of PKC),[21] gottlerin6976 (Go6976) (a specific inhibitor of Ca^{2+} dependent PKC-α/β), calphostin C (a specific inhibitor of Ca^{2+} dependent PKC-ɣ isoform),[22] rottlerin (a specific inhlbitor of Ca^{2+} independent PKC-Θ/δ), SB202190 (a inhibitor of p38 MAPK), radicicol (a inhibitor of Ras-Raf mediated signalling),[23,24] U0126 (a inhibitor of MEK1/2) and JNK inhibitor Ⅱ were purchased from calbiochem (San Diego, CA, USA). Specific antibodies (Abs) against phosphorylated (p)-p44/42, (p)-p38, (p)-JNK and control Abs were purchased from cell signaling technology (New England Biolabs, MA, USA).

3. Quantitation of Human IL-8 secretion

Cells were plated at 3×10^5 cells/mL in 6-well culture plates and stimulated with chitinase (10 and 100 ㎍/mL) for 1, 2, 4 or 7hr. The supernatants were harvested, and measured for IL-8 protein determinations by human IL-8 ELISA kit (R&D system, MN, USA) according to the manufacturer's instructions.

4. Quantitative RT-PCR

Total RNA was isolated using TRIzol®reagent (Invitrogen, Charlsbad, CA, USA) from H292 cells cultured with chitinase over specified induction times. For synthesis of first strand cDNA, RNA (2 ㎍) was incubated with 10 mM dNTP, 0.1 M DTT, 1 μL random hexamer (1 pmole), first strand buffer (100 mM Tris-HCl, pH 8.4, 250 mM KCl) and 1 μL superScript Ⅱ (200 U/μL) (Invitrogen, Charlsbad, CA) at 42℃ for 60 min, and then 70℃ 10 min. The PCR conditions for human IL-8 are 94℃ for 5 min and cycled 30 cycles at 94℃ for 30 sec, 56℃ for 30 sec and 72℃ for 30 sec, after which an final extension step at 72℃ for 5 min. Primer sequences were as follows: human IL-8: 5′-AGA TAT TGC ACG GGA GAA-3′ (sense) and 5′-GAA ATA AAG GAG AAA CCA-3′ (antisense); GAPDH: 5′-ACC ACA GTC CAT GCC ATC AC-3′ (sense) and 5′-TCC ACC ACC CTG TTG CTG TA-3′ (antisense)

5. Western immunoblot analysis

Cells were stimulated with chitinase in 0.5% FBS RPMI 1640 for specified induction times, and then were washed with cold PBS. The detached cells were lysed for 40 min on ice with lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 10% glycerol, and 0.5% NP-40). Protein samples were mixed with 5×SDS-PAGE buffer containing β-mercaptoethanol and heated at 95℃ for 5 min. Whole cell lysates (30 ㎍) were separated by 10% SDS-PAGE, then electro -transferred to nitro-cellulose membranes (Amersham International, Buckingham -shire, UK). The membranes were blocked for 1hr with 5% skim milk in TBST buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.2% Tween 20) and washed three times with TBST at room temperature. The blots were incubated overnight with specific antibody (1:1000) in 5% skim milk in TBST buffer at 4℃. After incubation with secondary antibody (1:2000), the signal was detected by the enhanced chemiluminescence method (ECL) (Amersham International, Buckinghamshire, UK).

6. Data analysis

The data were means ±SEM of at least three individual experiments. Statical analysis comparing between treatment and control groups was assessed by Student *t* test ($p < 0.05$ was considered significant).

Ⅲ. RESULT

1. Chitinase induces IL-8 production in a time and dose -dependent manner

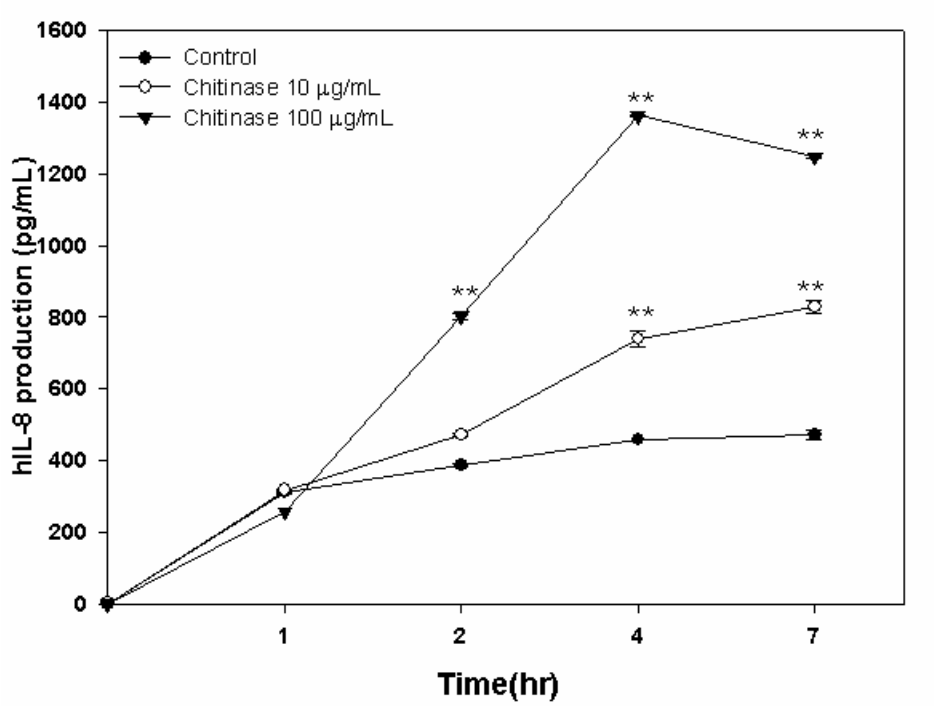
To examine whether chitinase induce IL-8 protein production and mRNA expression in human airway epithelial cells, cells were stimulated with different concentrations of *S. griseus* chitinase (10 and 100 ㎍/mL). As shown in Fig. 1 A, the production of IL-8 by H292 cells following stimulation with *S. griseus* chitinase was increased and showed maximal protein production at 4hr after stimulation. Based on 100 ㎍/mL *S. griseus* chitinase concentrations, IL-8 production was increased by 261.0 ± 7.1, 436.4 ± 8.8, 1247.7 ± 27.1, 1186.4 ± 10.0 pg/mL.

Additionally, the level of IL-8 mRNA expression was also elevated by *S. griseus* chitinase. However, the maximal mRNA expression was at 2hr after stimulation (Fig. 1. B).

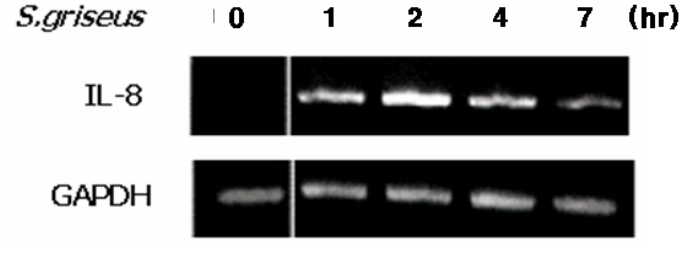
2. Role of MAPK in chitinase induced IL-8 production and mRNA expression

To investigate the chitinase induced MAPK activation, MAPK chemical inhibitors were treated and measured protein production and mRNA expression. Preincubation with SB202190 (75 μM) or U0126 (100 μM) resulted in inhibition of chitinase induced IL-8 protein and mRNA expression. However, JNK inhibitor Ⅱ (100 μM) failed to attenuate IL-8 production and mRNA expression (Fig. 2). In addition,

in the result of Western blot analysis, exposure to chitinase resulted in a increase in phosphorylated p44/42 and p38 MAPK level in H292 cells. After 5 min exposure, phosphorylations of p44/42 and p38 MAPK were detected. On the other hand, phospohorylation of JNK was not activated by chitinase, as shown in Fig. 3. C.

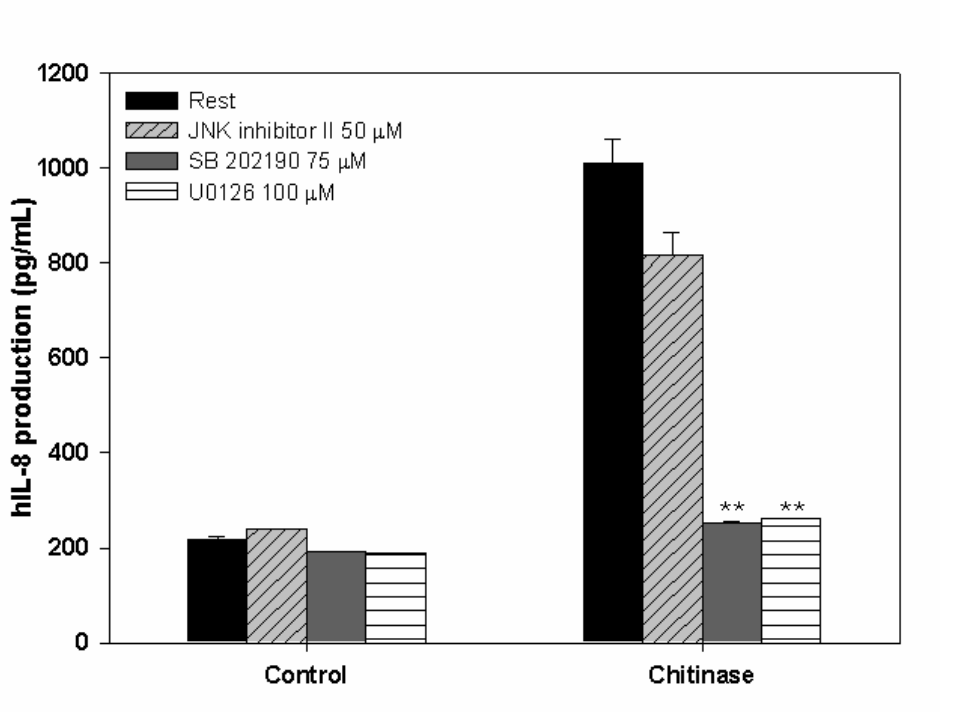


A

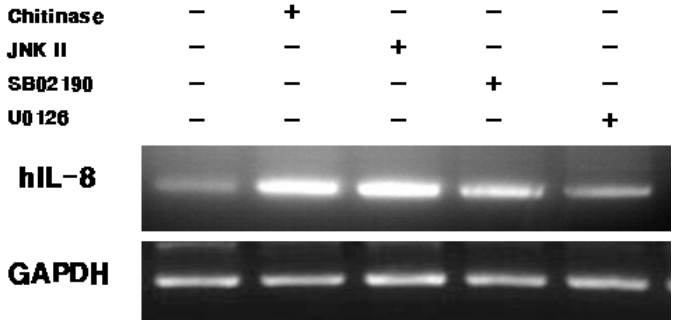


B

Fig. 1 The effect of chitinase on IL-8 production and mRNA expression (A) Cells were stimulated with different concentrations of *S. griseus* chitinase (10 and 100 ㎍/mL) at 37℃. The supernatants were collected and assayed for IL-8 by ELISA. ** $p < 0.05$ vs. control alone. The data represent the mean±SEM from four separate experiments. (B) The RNAs from cells treated with *S. griseus* chitinase were harvested and uesd for RT-PCR.


1200
1000
800
600
400
200
0
hIL-8 production (pg/mL)
Rest
JNK inhibitor II 50 μM
SB 202190 75 μM
U0126 100 μM
**
**
Control
Chitinase

A


Chitinase − + − − −
JNK II − − + − −
SB02190 − − − + −
U0126 − − − − +
hIL-8
GAPDH

B

Fig. 2 The effect of MAPK on *S. griseus* chitinase induced IL-8 production and mRNA expression. (A) Cells were preincubated with indicated concentrations of SB202190, U0126 and JNK for 1hr prior to stimulation with 100 ㎍/mL *S. griseus* chitinase. The supernatants were collected and evaluated for IL-8 production by ELISA. *** $p<0.001$ vs. chitinase alone, ** $p<0.01$ vs chitinase alone, * $p<0.05$ vs chitinase alone. The data represent the mean±SEM from four separate experiments. (B) The RNAs from cells treated with MAPK inhibitors and *S. griseus* chitinase were harvested and uesd for RT-PCR.

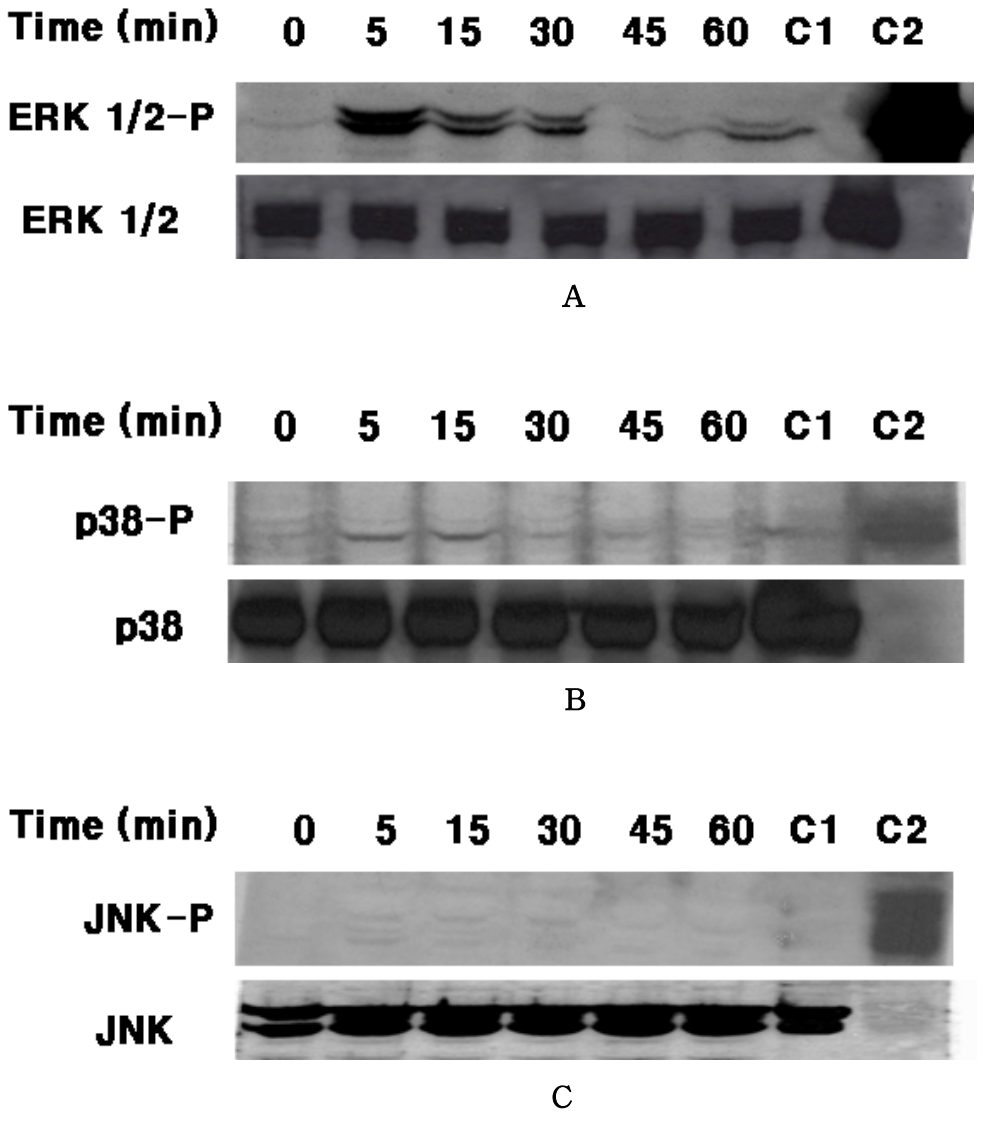
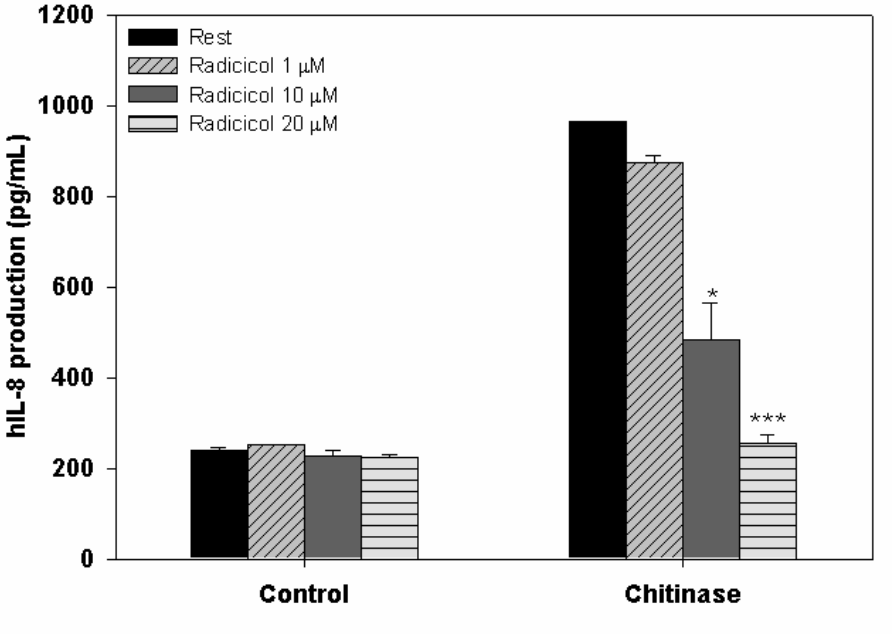


Fig. 3 Phosphorylation assays of MAPK activities. (A) Cells treated with chitinase were harvested at the indicated time points and then lysed. The equal amounts of cell extracts were resolved on 10% acrylamide gels and then subjected to western blot analysis. C1: non-phosphorylated control, C2: phosphorylated control.

3. Role of Ras-Raf in chitinase induced IL-8 production and mRNA expression

To demonstrate the role of Ras-Raf in chitinase-induced IL-8 production, cells were preincubated with radicicol for 1hr prior to stimulation with chitinase. At a concentration of 20 μM, radicicol led to a significant inhibition of chitinase induced IL-8 protein and mRNA expression (Fig. 4).



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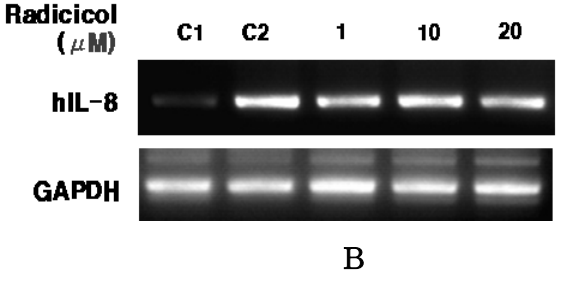

B

Fig. 4 Dose-dependent effect of Ras-Raf inhibitor on *S. griseus* chitinase induced IL-8 production and mRNA expression. (A) Cells were preincubated with indicated concentrations of radicicol for 1hr prior to stimulation with 100 ㎍/mL *S. griseus* chitinase. The supernatants were collected and evaluated for IL-8 presence. *** $p<0.001$ vs. chitinase alone, ** $p<0.01$ vs chitinase alone, * $p<0.05$ vs chitinase alone. The data represent the mean±SEM from four separate experiments. (B) The RNAs from cells treated with radicicol and *S. griseus* chitinase were harvested and uesd for RT-PCR.

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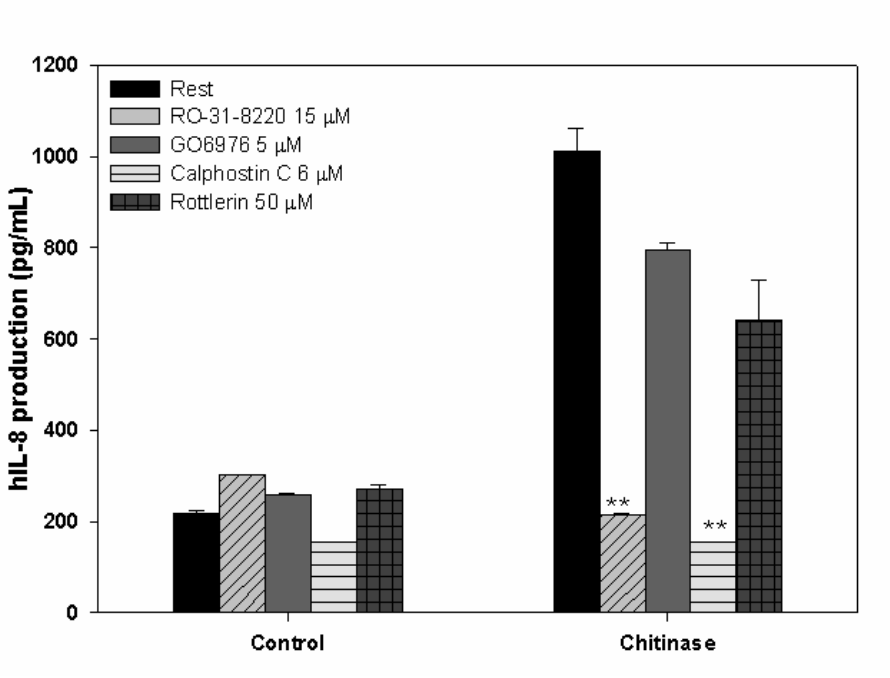
4. Role of PKC in chitinase induced IL-8 production and mRNA expression

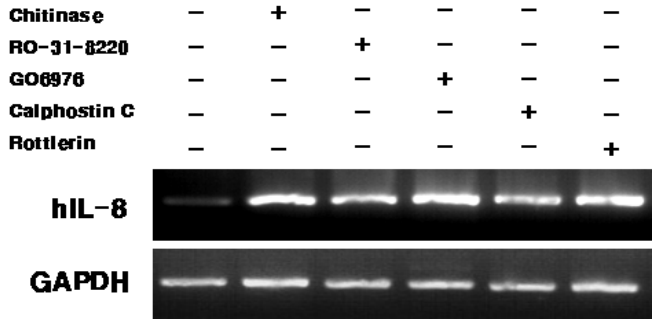
To examine the role of PKCs in chitinase induced IL-8 production, cells were treated with Ro-31-8220, Go6976, calphostin C or rottlerin for 1hr prior to stimulation with chitinase. As shown in Fig. 5, both Ro-31-8220 (15 μM) and calphostin C (6 μM) reduced chitinase induced IL-8 production completely and IL-8 mRNA expression.

5. Role of Ca^{2+} in chitinase induced IL-8 production and mRNA expression

To evaluate the role of intracellular Ca^{2+} in chitinase induced IL-8 production, cells were preincubated with indicated concentrations of BAPTA/AM, a intracellular Ca^{2+} chelator. As shown Fig. 6, BAPTA/AM inhibited chitinase induced IL-8 production and IL-8 mRNA expression in dose-dependent manner.

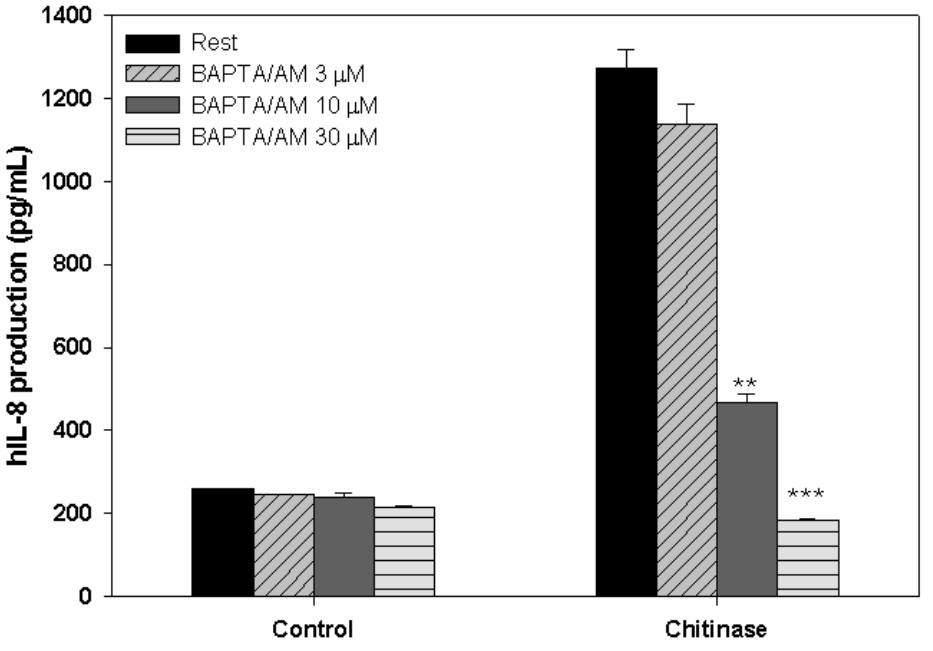

1200
1000
800
600
400
200
0
hIL-8 production (pg/mL)
Rest
RO-31-8220 15 μM
GO6976 5 μM
Calphostin C 6 μM
Rottlerin 50 μM
**
**
Control
Chitinase

A

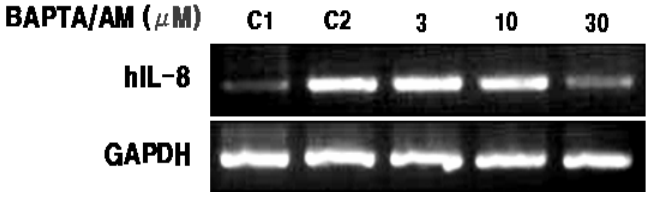

Chitinase – + – – – –
RO-31-8220 – – + – – –
GO6976 – – – + – –
Calphostin C – – – – + –
Rottlerin – – – – – +
hIL-8
GAPDH

B

Fig. 5 The effect of PKC inhibitors on *S. griseus* chitinase induced IL-8 production and mRNA expression. (A) Cells were preincubated with indicated concentrations of Ro-31-8220, Go6976, calphostin C or rottlerin for 1hr prior to stimulation with 100 μg/mL *S. griseus* chitinase. The supernatants were collected and evaluated for IL-8 presence. *** $p<0.001$ vs. chitinase alone, ** $p<0.01$ vs chitinase alone, * $p<0.05$ vs chitinase alone. The data represent the mean±SEM from four separate experiments. (B) The RNAs from cells treated with PKC inhibitors and *S. griseus* chitinase for the indicated concentrations were harvested and uesd for RT-PCR.


1400
1200
1000
800
600
400
200
0
hIL-8 production (pg/mL)
Rest
BAPTA/AM 3 μM
BAPTA/AM 10 μM
BAPTA/AM 30 μM
**
***
Control
Chitinase

A


BAPTA/AM (μM)
C1
C2
3
10
30
hIL-8
GAPDH

B

Fig. 6 Dose-dependent effect of intracellular Ca^{2+} on *S. griseus* chitinase induced IL-8 release and mRNA expression. (A) Cells were preincubated with indicated concentrations of BAPTA/AM for 1hr prior to stimulation with 100 ㎍/mL *S. griseus* chitinase. The supernatants were collected and evaluated for IL-8 production. *** $p<0.001$ vs. chitinase alone, ** $p<0.01$ vs chitinase alone, * $p<0.05$ vs chitinase alone. The data represent the mean±SEM from four separate experiments. (B) The RNAs from cells treated with BAPTA/AM and *S. griseus* chitinase were harvested and uesd for RT-PCR.

Ⅳ. Discussion

The chitinase is produced by a variety of organims such as insect, parasite, fungus, bacteria, and human.[1,28] Even though we are exposed to them daily life, the mechanism of exogenous chitinase mediating lung inflammatory responses has not fully understood. Among many organisms with ability to produce exogenous chitinase, bacteria secretes many known extracellular proteins like chitinase, lecithinase, hemolysin and lipase. And these proteins can activate host inflammatory responses.[28] *Streptomycetes* species, mycelia- and spore forming gram positive bacteria, has known to enrich anti-bacterial and anti-fungal compounds like chitinase for hydrolysis of macromolecules including chitin. Especially, *S. griseus* is easily collected from indoor air and walls.[17,18]

The pathogenesis of IL-8 in a variety of acute and chronic lung diseases has been emphasized for a long time.[27] IL-8 is a potent chemoattractant for neutrophils and plays a major role in lung inflammatory diseases. In patients with asthma, IL-8 is increased in BAL fluid.[27] In this study, we investigated the effect of chitinase on IL-8 expression in human bronchial epithelial cells. We found that chitinase induced IL-8 protein secretion as well as mRNA expression. The MAPK has been shown to regulate IL-8 expression in number of cell types, including lung epithelial cell.[25] We demonstrated MAPK activity related with chitinase induced IL-8 production. At first, inhibition of the JNK signalling pathway by the pharmacological

inhibitor JNK inhibitor II failed to block chitinase induced IL-8 production. In contrast, U0126 (a inhibitor of ERK 1/2) and SB202190 (a inhibitor of p38 MAPK) prevented completely chitinase induced IL-8 production by ERK and p38 MAPK, suggesting a role for these two MAPK in regulating its expression and secretion.

In addition, we demonstrated Ras-Raf mediated signalling pathway which has been known to MAPK cascade upstream. Pretreatment with radicicol is almost completely attenuated protein secretion and mRNA expression of chitinase induced IL-8 in dose dependent manner. Because radicicol has been known to be an inhibitor for Ras-Raf mediating pathway by distabilizing Raf kinase.

Several cellular responses in IL-8 has been mediated via PKC signal transduction.[27] We demonstrated that chitinase induced IL-8 production is also regulated by a PKC-dependent pathway. Chitinase induced IL-8 was completely suppressed by the pan-PKC inhibitor RO-31-8220. Additionlly, we examined a requirement for the specific PKC isoforms in chitinase induced IL-8 production. As a result, cells treated with calphostin C, a PKC γ specific inhibitor, showed a markedly reduced chitinase induced IL-8 expression. In contrast, inhibition of PKC α, β and δ by GO6976 and rottlerin had no effect on IL-8 protein secretion and mRNA expression. These data show that the PKC γ isoform takes part in chitinase induced IL-8 production. Previous studies demonstrated that activation of PKC γ induce IL-8 expression in response to TPA and thrombin.[29,30]

A number of studies have provided evidence that PKC affects Ca^{2+}

levels in a number of cell types via induction of specific Ca^{2+} channels.[14,15,20] The present study also investigated whether intracellular Ca^{2+} contribute to chitinase induced IL-8 production by BAPT/AM, a intracellular Ca^{2+} chelator. As a result, BAPT/AM reduced completely chitinase induced IL-8 production.

In conclusion, we have demonstrated the signalling pathway leading to the up regulation of IL-8 in bronchial epithelial cell in response to the inflammatory stimulus *S. griseus* chitinase. This work identified Ca^{2+} dependent PKC ɣ involved, confirmed the Ca^{2+} contribution and demonstrated the requirement for ERK and p38 MAPK activation. Further studies are necessary to reveal the involvement of transcription factors in *S. griseus* chitinase induced IL-8 production.

Ⅴ. CONCLUSION

1. *S. griseus* chitinase incresed IL-8 in human airway epithelial cells.
2. Activation of ERK, p38 MAPK and Ras-Raf increased *S. griseus* chitinase- induced IL-8
3. PKC isoform ɣ involved in *S. griseus* chitinase-induced IL-8 production in human airway epithelial cells.

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ABSTRACT(In Korean)

인체 기관지 상피세포에서 chitinase에 의한 Interleukin-8 분비에서 MAP kinase의 역할 규명

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홍 정 연

Chitinase는 다양한 chitin 포함 유기체로부터 생산된다. 최근 몇몇 연구에서 acidic mammalian chitinase (AMCase)가 Th2 매개 airway inflammation을 유도시킨다고 보고하고 있다. 그러나 우리 주변에서 자주 접하게 되는 외부 chitinase에 대한 면역반응 기작은 알려진 것이 없다. 또한 여러 가지 자극에 의해 만들어지는 cytokine인 IL-8은 기관지 천식의 allergic inflammation의 발병에 역할을 한다고 알려져 있다. 본 연구에서는 *Streptomyces griseus* (*S. griseus*)로부터 추출한 외부 chitinase가 인간 호흡기 상피세포에 IL-8 생산을 유도하는지 protein kinase와의 관계를 통하여 밝히고자 하였다.

배양한 호흡기 상피세포에 다양한 농도와 시간으로 *S. griseus* chitinase를 처리해주고, IL-8 발현량은 enzyme-linked immuno sorbent assay (ELISA)와 reverse transcriptase polymerase chain reaction (RT-PCR)으로 측정하였다. 또한 다양한 inhibitor를 사용하

여 *S. griseus* chitinase에 의한 IL-8 발현 기작을 밝히고자 하였다.

H292 세포에 *S. griseus* chitinase를 처리해 주었을 때 IL-8 생성과 mRNA 발현이 시간과 농도에 따라 증가되었다. 또한 ERK 1/2와 p38 MAPK inhibitor인 U0126과 SB202190을 처리해 주었을 때 IL-8의 생성이 H292 세포에서 의미있게 감소하였으나 JNK inhibitor에 의한 의미있는 감소는 보이지 않았다. 그리고 Calphostin C 와 Ro-31-8220을 처리를 통해 PKC 아형 γ의 활성화가 chitinase에 의한 IL-8 생성에 필요하다는 것을 확인할 수 있었다. 또한 Radicicol의 처리를 통해 Ras-Raf signalling도 chitinase에 의한 IL-8 생성을 조절할 수 있음을 알 수 있었다.

결론적으로 *S. griseus* chitinase는 인간 호흡기 상피세포에서 PKC, Ras-Raf, ERK, p38 MAPK의 활성화에 의해 IL-8 의 생성을 유도할 수 있을 것으로 사료된다.

핵심되는 말: chitinase, 호흡기 상피세포, IL-8, ERK, p38 MAPK, PKC