

사람 정상 코점막 상피세포에서 사이토카인에 의한 MUC8과 MUC5AC의 발현 및 조절

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Expression and Regulation of MUC8 & MUC5AC by Various Cytokines in Normal Human Nasal Epithelial Cells

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ABSTRACT

Background and Objectives : Sinusitis is one of the most commonly reported diseases in the world. A network of inflammatory mediators is known to be involved in the pathogenesis of chronic sinusitis and nasal mucus secretion may also be under the control of an inflammatory mediator network. To date, 12 human mucin genes have been identified ; however, the regulation of MUC8 has not yet been found out. In this study, we described the regulation of the MUC8 mRNA expression by inflammatory mediators and investigated its cellular location. **Materials and Method :** MUC8 mRNA and MUC5AC mRNA were detected in culture using passage-2 normal human nasal epithelial (NHNE) cells after the treatment with a mixture of following inflammatory mediators ; TNF- α , IL-1 β , LPS, IL-4, PAF. The translocation of MUC8 mRNA from the nucleus to the cytoplasm was investigated by treating the inflammatory mediators with in situ hybridization. **Results :** We found that a mixture of inflammatory mediators increased the MUC8 mRNA expression but decreased the MUC5AC mRNA expression in cultured normal human nasal epithelial cells. Among the inflammatory mediators, Interleukin-4 was responsible for the decrease in the MUC5AC mRNA expression and the MUC5AC mucin secretion. We also found that MUC8 mRNA resides in the nucleus of goblet cells and is transported into the cytoplasm following the treatment with inflammatory mediators. **Conclusion :** These results indicate that MUC8 may play an important role in the pathogenesis of mucus hypersecretion in chronic sinusitis. (Korean J Otolaryngol 2001;44:600-5)

KEY WORDS : Normal human nasal epithelial cells · Mucin · Cytokines · Mucin gene 8 (MUC8) · Mucin gene 5AC (MUC5AC).

TNF- α , IL-4, IL-5, IL-6, IL-8, GM-CSF, (autocytotoxicity),

2001 1 16 / 2001 4 17
: 120-749 134

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12 (MUC1 - 4, MUC5AC, MUC5B, MUC6 - 9, MUC11, MUC12)가 MUC2, MUC5AC, MUC5B, MUC7 MUC8 MUC2 MUC5AC mRNA MUC5B MUC7 MUC8 mRNA가 hybridization MUC8 mRNA MUC2 mRNA TNF - endotoxin MUC5AC mRNA IL - 4, platelet - activating factor(PAF), IL - 9 MUC8 MU - C8 mRNA가 가 in situ hybridization MUC8 mRNA MUC8 가 , in - vivo in - vitro 가 TNF - , IL - 1 , LPS, PAF, IL - 4 in - vitro MUC8 mRNA 가 MUC5AC Air - liquid interface culture(ALI) 10⁵ (passage - 2) ba - sal epithelial growth medium(BEGM) DMEM 1 : 1 가 가 (24.5 mm, 0.45 μ m pore size ; Transwell - clear, Cos - tar Corp., Cambridge, MA, USA) 9 ALI

37 , 5% CO₂ 11 5 (IL - 1 10 ng/ml, TNF - 10 ng/ml, lipo - polysaccharide(LPS) 5 μ g/ml, plateletactivating factor (PAF) 10⁻⁸M, IL - 4 100 ng/ml) 8, 24, 48 total RNA total RNA dot blotting (a generous gift from Dr. Davis, University of North Carolina, NC, USA) H6C5(1 : 1000, a generous gift from Dr. Davis, University of North Carolina, NC, USA) horseradish peroxidase conjugated goat anti - mouse IgG , chemiluminescence(ECL kit, Amersham, Buckinghamshire, UK) . Standard curve linear regression analysis ± , st - udent 's t - test Total RNA, nuclear RNA cytoplasmic RNA Total RNA Trir - eagent(Molecular Research Center. Cincinnati, OH, USA) . RNA 1 μ g RNA ethi - dium bromide 6.6% formaldehyde 1.5% agarose gel RNA Buffer RLN(50 mM TrisCl, pH8.0/140 mM NaCl/ 1.5 mM MgCL₂/0.5% NP - 40) 175 μ l 1.5 ml tube 5 4 300 g 2 Trireagent RNeasy Mini Kit(Qiagen, Valencia,

MUC8 MUC5AC mRNA

Calif., USA) RNA .

MUC5AC mRNA MUC8 mRNA RT - PCR

Guzman ¹⁶⁾ RT - PCR . Oligo - nucleotide primers MUC5AC cDNA (Genbank #U06711) , 5 'primer, TCCGGCCTCA - TCTTCTCC 3 'primer, ACTTGGGCACTGGTGCTG 680 bp , MUC8 cDNA Genbank #U14383) 5 'primer, AC - AGG - GTTTCTCCTCATTG 3 ' primer, CGTTTATTCC - AGCACTGTTC 239 bp MUC5AC MUC8 mRNA RT - PCR . RT - PCR 2 microglobulin(335 bp, Clontech Lab.) mRNA comparative kinetic analysis ¹⁶⁾ PCR 50 ng/ml ethidium bromide 2% Sea - kem agarose gel(FMC, Rockland, ME) . PCR linear range PCR cycle genomic DNA RT - PCR PCR reverse transcriptase RT - PCR PCR

가 가 MUC - 5AC MUC8 가 , 5 가 24 MUC5AC mRNA , MUC8 mRNA MUC5AC

Cytospin slide MUC8 in situ hybridization Cytospin slides 4% MUC8 antisense sense RNA probes Sp6 T7 RNA polymerases in vitro transcription (forward - ACAGGGTTTCT - CCTCATTG, reverse - CGTTTATTCCAGCACTGTTC, EMBL Accession number U14383). Cytospin slides 1 ug/ml DIG - labelled cRNA 56 . Hybridization buffer 50% formamide

5 x sodium chloridesodium citrate(SSC), 40 μg/ml ssDNA가 . Hybridization 1 2 x SSC 0.1 x SSC anti - DIG Fab

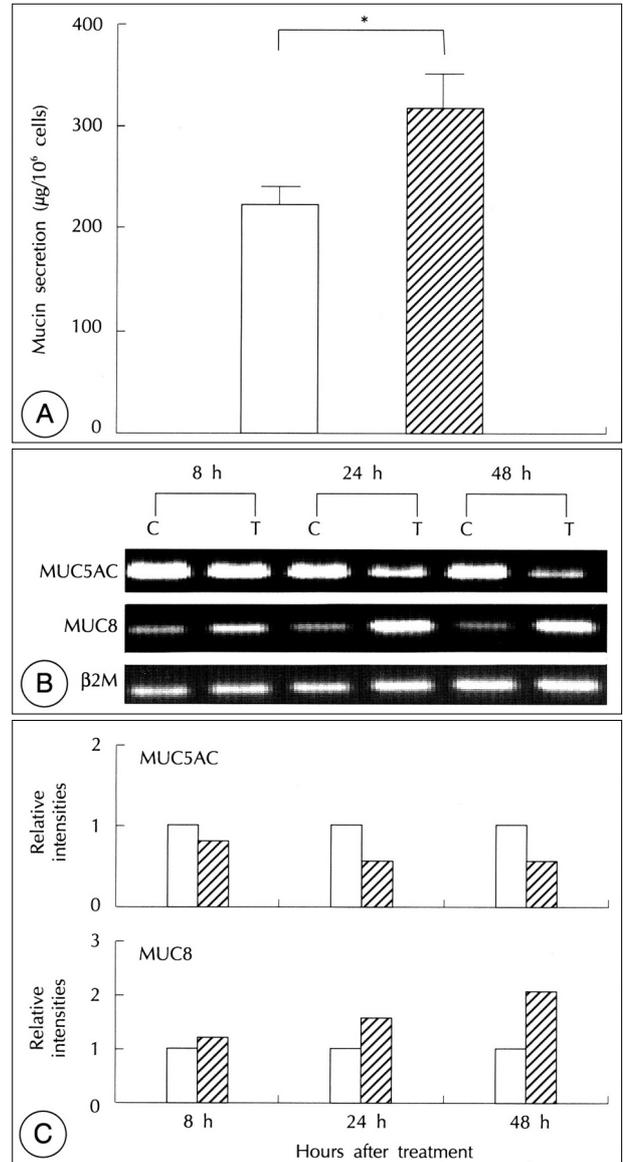


Fig. 1. Effects of treatment with a mixture of inflammatory mediators including TNF- α , IL-1 β , LPS, IL-4 and PAF on mucin secretion, MUC5AC mRNA, and MUC8 mRNA in cultured normal human nasal epithelial cells. The data shown in Figure 1A is representative of results obtained in three separate experiments. The mucin secretion 24 h after the treatment showed a 40% increase (Fig. 1A, the mixture of inflammatory mediators : 313.7 \pm 29.3 μ g/10⁶ cells ; control : 223.1 \pm 18.6 μ g/10⁶ cells). Expression of MUC5AC mRNA gradually decreased after 24 h treatment. However, expression of MUC8 mRNA significantly increased after 8 h of treatment and gradually increased over time (Fig. 1B). After treatment with a mixture of the five inflammatory mediators (Fig. 1C), the relative intensity of MUC5AC mRNA showed a 50% decrease over time, and that of MUC8 mRNA showed a 2.1-fold increase compared to the control gene. White bars indicate control (C) and black bars demonstrate treatment (T) with inflammatory mediators.

2 . Nitroblue tetrazolium chloride (NBT) and 5 - bromo - 4 - chloro - 3 - indolylphosphate (BCIP)

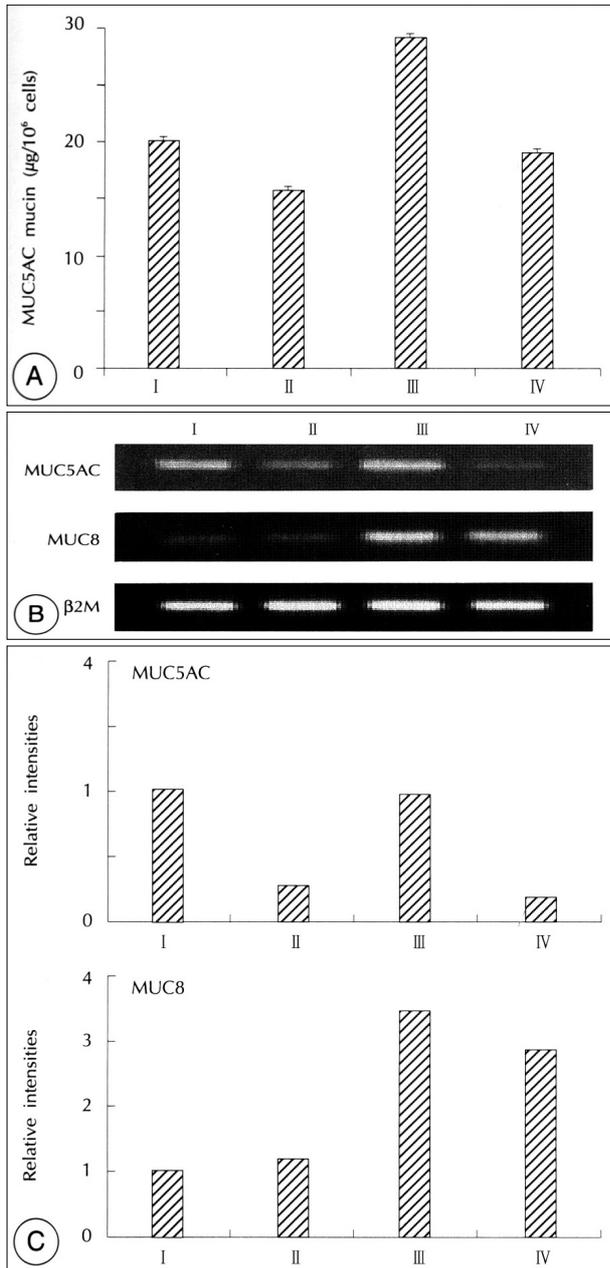


Fig. 2. Effects of IL-4 alone, the deletion of IL-4 from the mixture of inflammatory mediators, and treatment with the mixture of inflammatory mediators on MUC5AC mRNA and mucin secretion in cultured normal human nasal epithelial cells. Treatment with either IL-4 alone or the mixture of inflammatory mediators decreased MUC5AC mucin secretion (Figure 2A, control () : $20.3 \pm 0.3 \mu\text{g}/10^6$ cells, IL-4 alone () : $16.1 \pm 0.2 \mu\text{g}/10^6$ cells, deletion of IL-4 from a mixture () : $28.8 \pm 0.3 \mu\text{g}/10^6$ cells, a mixture of inflammatory mediators () : $18.4 \pm 0.2 \mu\text{g}/10^6$ cells) and MUC5AC mRNA expression (Fig. 2B and 2C) compared to the control.

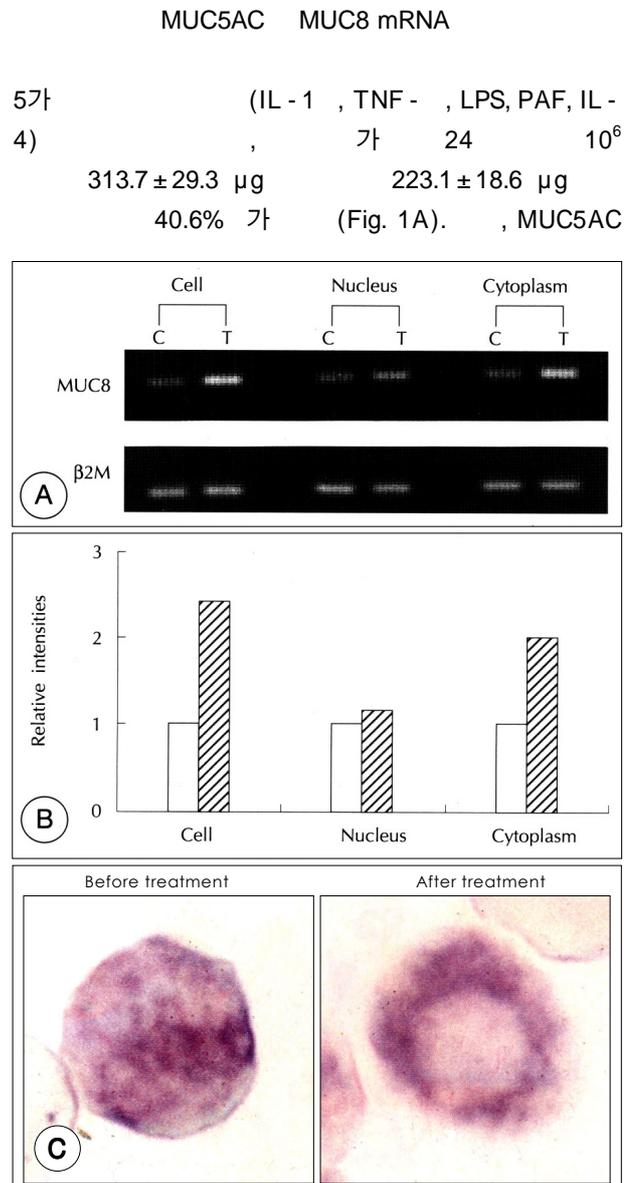


Fig. 3. Effects of the mixture of inflammatory mediators on expression and translocation of MUC8 mRNA in the nucleic and cytoplasmic RNA. Interestingly, the expression of nucleus MUC8 mRNA did not increase significantly but that of cytoplasmic MUC8 mRNA strongly increased. This result suggests that the increased levels of total cellular MUC8 mRNA is due to the increased levels of MUC8 in the cytoplasm, which is translocated from the nucleus (Fig. 3A, 3B). This finding is further supported by the observation that signals of MUC8 messages were strongly intensified in the cytoplasm of cells treated with inflammatory mediators as determined by in situ hybridization (Fig. 3C). White bars indicate control (C) and black bars demonstrate treatment (T) with inflammatory mediators.

MUC8 MUC5AC mRNA

mRNA 24 가 MUC5AC mRNA IL - 4,¹²⁾ ne -
50% MUC8 mRNA 8 가 NA utrophil elastase,¹⁷⁾ ,
48 2 가 (Fig. 1B and C). endotoxins¹⁸⁾ MUC5AC
mRNA 가
IL - 4 IL - 4 5가 MUC5AC MUC5AC MUC5AC 19) ,
IL - 4 MUC5AC mRNA MUC5AC Fig. 2 IL - 4 MUC5AC
(Fig. 2). , IL - 4 MUC8 mRNA mRNA 가 IL - 4
가 (Fig. 2B and 2C). (NHTBE cell) IL - 4
MUC8 mRNA MUC5AC MUC5B mRNA
5가 MUC5AC mRNA IL - 4²⁰⁾
MUC8 mRNA
5가
MUC8 mRNA RT - PCR *in situ* hybridization MUC8 mRNA가 가 in situ hy -
NA MUC8 mRNA 가가 24 MUC8 mRNA가 MUC8 mRNA가
total RNA 가 가
MUC8 mRNA 가가
(Fig. 3A and 3B). *in situ* hybridization MUC8 mRNA가
MUC8 mRNA가
mRNA가 가 (Fig. 3C). MUC8
5가
MU - C8
mRNA RT - PCR *in situ* hybridization MUC8 mRNA
가 가 MUC8 mRNA
MUC8 mRNA 가
total RNA
가 MUC8 mRNA
가가 *in situ* hybridization
TNF - , neutrophil elastase, IL - 4, IL - 9, en -
dotoxin 가 MUC8 mRNA가 가
MU -
C8 mRNA 5가 MUC8 mRNA
(NHNE cell) MUC8 mRNA 가 NHNE cell
MUC8 mRNA 8 MUC5AC mRNA 가 MUC8
MUC8 mRNA 가 48 mRNA 가
가 MUC8

MUC5AC

MUC5AC

가

, MUC8

가

가

가

C8 mRNA

가

MUC8 mRNA

가

MUC8

NHNE cell MU -

MUC5AC mRNA

(mucus hypersecretion)

MUC8 · MUC5AC.

2000 (2000 - 20)

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