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Mucin 5 Subtype AC Expression and Upregulation in the Nasal Mucosa of Allergic Rhinitis Rats

Weiwei Wang, MD¹, and Ming Zheng²

No sponsorships or competing interests have been disclosed for this article.

Abstract

Objective. To elucidate the functions of nuclear factor kappa B (NF- κ B) in mucin hypersecretion in allergic rhinitis (AR), we examined the *in vivo* effects of an NF- κ B inhibitor, ammonium pyrrolidine dithiocarbamate (PDTC), on mucin 5 subtype AC (*MUC5AC*) expression in the nasal mucosa of ovalbumin-sensitized rats.

Study Design. Randomized animal study.

Setting. Academic medical center.

Subjects and Methods. Sprague Dawley rats were randomized into a control group (group A), an AR model group (group B), and an AR model treated with an NF- κ B inhibitor (group C). Rats in groups B and C were sensitized systemically and locally by ovalbumin injection and inhalation, whereas group A was treated with normal saline in place of ovalbumin. Pyrrolidine dithiocarbamate (100 mg/kg/d) was given to group C by intraperitoneal injection for 5 days. NF- κ Bp65, *MUC5AC*, tumor necrosis factor (TNF)- α , and interleukin (IL)-6 were detected by immunohistochemistry, Western blotting, enzyme-linked immunosorbent assay, or real-time polymerase chain reaction.

Results. NF- κ B was activated in group B, and significant NF- κ Bp65 protein was expressed in the nucleus of cells from the nasal mucosa, resulting in upregulated transcription from TNF- α and IL-6 genes, as well as increased contents of TNF- α and IL-6 in the nasal lavage fluids. Pyrrolidine dithiocarbamate inhibited nuclear localization of NF- κ Bp65 and subsequent downregulation of the transcription and secretion of TNF- α and IL-6. *MUC5AC* was upregulated in group B but reduced in a time-dependent manner after inhibition of NF- κ B activation.

Conclusion. NF- κ B activation might induce *MUC5AC* hypersecretion in AR rats by inflammatory cytokines TNF- α and IL-6.

Keywords

allergic rhinitis, nuclear factor kappa B, mucin 5 subtype AC, tumor necrosis factor- α , interleukin-6

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Allergic rhinitis (AR) results from an immunoglobulin E-mediated allergy associated with nasal inflammation of variable intensity and is a condition seriously affecting the patients' quality of life. Over the past 20 years, the incidence of AR has been rising.¹

Homeostasis of mucus production plays an essential role in proper mucociliary and innate immune function in the airway.² To date, 11 types of mucin genes (mucin 1, 2, 3, 4, 5 subtype AC, 5 subtype B, 6, 7, 8, 13, and 19) have been described as being expressed in the airway.³ The MUC 5 subtype AC (*MUC5AC*) of these is generally recognized to be the major airway mucin.⁴ The main characteristics of AR are airway inflammation and mucus hypersecretion.^{5,6} Under disease conditions, the excessive production of mucin can break the fluid balance of the airway by obstructing mucociliary clearance, resulting in obstruction and infection. Thus, understanding the mechanism of *MUC5AC* regulation may lead to new therapies for the treatment of aberrant mucus production in AR.

Nuclear factor kappa B (NF- κ B) is a protein with polytranscriptional regulatory roles, which plays a pivotal role in inflammatory and immune responses by regulating immune and inflammation-related cytokine and inflammatory mediator genes. It also plays a vital role in the initiation and perpetuation of allergic inflammation.^{7,8} Although many studies have shown that NF- κ B has a critical role in AR,^{9,10} information regarding the involvement of NF- κ B in mucin hypersecretion in AR is limited. Only a few studies have demonstrated the involvement of NF- κ B in *MUC5AC* upregulation by lipoproteins of *Haemophilus influenzae* or mycoplasma pneumonia.^{11,12} The information regarding *MUC5AC* regulation is only at the cellular level and has not been extended to AR disease.

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In this study, we established an AR model that was treated with pyrrolidine dithiocarbamate (PDTC), an NF- κ B inhibitor, to determine the role of NF- κ B in the regulation of *MUC5AC* in the nasal mucosa of AR rats. Description of this pathway from this study could provide a theoretical basis to study mucin hypersecretion in AR.

Materials and Methods

Animals

This study was performed in accordance with the accepted policy on the use of animals, and all the procedures were approved by the government animal care authorities. One hundred Sprague Dawley rats, 250 to 300 g (Slaccas, Shanghai, China), were randomized into 3 groups: control (group A, 20 animals), model (group B, 20 animals), and AR model with NF- κ B inhibitor (group C, 60 animals). According to 1 day, 3 days, or 5 days of treatment, group C was subdivided into groups C1, C2, and C3 of 20 rats each, respectively. Each group was composed of 20 rats (immunohistochemistry, 5 rats; Western blotting, 5 rats; real-time polymerase chain reaction [PCR], 5 rats; and enzyme-linked immunosorbent assay [ELISA], 5 rats). Before this experiment, we performed a preliminary experiment. On the basis of the data from the preliminary experiment, the sample capacity was estimated. The equation used was the following:

$$n = 2 \times [(t_{\alpha} + t_{\beta}) \times \sigma/\delta]^2,$$

where $\alpha = 0.05$, $\beta = 0.10$, $t_{\alpha} = 1.96$, and $t_{\beta} = 1.282$. The result showed the number of samples in each group had statistical significance.

The establishment of the AR model followed a published protocol.¹ Rats in groups B, C1, C2, and C3 were sensitized systemically by intraperitoneal injection of 1 mL saline containing 0.3 mg ovalbumin (Sigma, St Louis, Missouri) and 300 mg $\text{Al}(\text{OH})_3$ once every other day for 14 days. They were then sensitized locally by dripping 0.1 mL saline containing 10 mg ovalbumin into the bilateral nasal cavities once a day from the 15th to the 21st day; group A was treated with normal saline without ovalbumin. On the 21st day after inhalation of antigen or saline, groups A and B were sacrificed by an overdose of pentobarbitone (100 mg/kg), whereas group C was injected intraperitoneally with 100 mg/kg ammonium PDTC (Sigma) for 5 days. The rats from groups C1, C2, and C3 were respectively sacrificed on the first, third, and fifth days after PDTC treatment.

For the collection of nasal lavage fluids (NLF), an epidural anesthesia tube was placed and fixed into the nasal cavity at 2 cm, the rats were placed upside down, and the bilateral nasal cavities were lavaged with 1.5 mL sterile normal saline. The collected NLF was centrifuged at 3000 rpm/min for 15 minutes, and the supernatant was stored at -70°C . The nasal mucosa tissues of the rats were collected, and one part was stored at -80°C and the other was fixed in a 4% paraformaldehyde solution at pH 7.4 overnight at 4°C and then embedded in paraffin.

Immunohistochemistry

Paraffin-embedded nasal mucosa were sectioned at 5 μm . Sections were mounted on poly-L-lysine-coated slides, deparaffinized, and then rehydrated through an ethanol gradient. Intrinsic peroxidase activity was blocked with H_2O_2 for 10 minutes. The slices were blocked with nonimmune serum for 10 minutes and then incubated overnight at 4°C with a 1:250 dilution of mouse anti-*MUC5AC* antibody (Abcam, Cambridge, Massachusetts) or a 1:200 dilution of rabbit anti-NF- κ Bp65 antibody (Abcam). Sections were incubated for 10 minutes with the biotin-conjugated secondary antibody followed by incubation with horseradish peroxidase (HRP)–streptavidin for 10 minutes. A magnification of $\times 400$ was used to define 20 microscope fields per section, and the results were photographed with a digital camera. Analysis of the images was carried out with Image-Pro Plus 12.0 (Media Cybernetics, Rockville, Maryland), which had been programmed to measure the integral optical density (OD) of the immunohistochemically positive areas, and the mean OD (MOD) was obtained. Two representative sections from each rat were measured.

ELISA

The amount of tumor necrosis factor (TNF)- α or interleukin (IL)-6 in the NLF was detected by the ELISA kit (R&D Systems, Minneapolis, Minnesota). The procedure was as follows: standard sample was diluted to 7.5, 15, 30, and 60 ng/L, with blank, standard, and sample wells in enzyme-coated plates. A 50- μL standard sample was added into standard wells. Then, 40 μL sample dilution and 10 μL sample were added into sample wells followed by incubation at 37°C for 30 minutes. Next, 50 μL of HRP-conjugated anti-TNF- α or anti-IL-6 monoclonal antibody was added to wells, except for blank wells, followed by incubation at 37°C for 30 minutes, and then 50 μL of developer A and developer B was added followed by incubation at 37°C for 10 minutes. Then, 50 μL of stop buffer was added into the wells. Optical density values were measured at 450 nm by an ELISA plate reader (Bio-Rad, Hercules, California).

Western Blotting

Nucleoprotein and cytoplasmic protein were isolated from mucosa samples using a Nuclear-Cytosol Extraction Reagent Kit (Pierce, Rockford, Illinois). Nucleoprotein was used to detect NF- κ Bp65, and cytoplasmic protein was used to detect *MUC5AC*. After denaturation, equal amounts of protein from each sample were prepared for 12% sodium dodecyl sulfate (SDS) gel electrophoresis. Proteins were transferred after electrophoresis to PVDF membranes (Millipore, Billerica, Massachusetts). The membranes were blocked with blocking buffer (Beyond, Jiangmen City, Jiangsu Province, China) for 3 hours and then were incubated overnight at 4°C with a 1:300 dilution of mouse anti-*MUC5AC* antibody, a 1:500 dilution of mouse anti-NF- κ Bp65 antibody, a 1:1000 dilution of mouse anti- β -actin antibody (Abcam), or a 1:300 dilution of anti-histone H2A-1 antibody (Abcam). The membranes

were incubated for 1 hour with the corresponding HRP-conjugated secondary antibody, and antibody binding was detected with Beyond ECL Plus. The density of the bands in the Western blot was analyzed using Quantity One software (Silk Scientific Corporation, Orem, Utah).

Real-time PCR

Total RNA from the nasal mucosa was isolated by TRIzol Reagent (Invitrogen, Carlsbad, California). One microgram of total RNA was reverse transcribed to cDNA using the RevertAid First Strand cDNA Synthesis Kit (Fermentas, Burlington, Ontario, Canada). Real-time PCR was performed using the Platinum SYBR Green qPCR SuperMix-UDG kit (Invitrogen) and a real-time thermal cycler (ABI 7500; Applied Biosystems, Foster City, California). The primers are as follows:

β -actin: 5'-accgtgaaagatgaccagat-3',
 5'-agctgtggtggtgaagctgtag-3'
 TNF- α : 5'-gctcagaacacacagagatgct-3',
 5'-ccggcctccaataatacat-3'
 IL-6: 5'-ccacttcacaagtcggaggctta-3',
 5'-gtgcatcatcgctgttcatacatc-3'
 MUC5AC: 5'-gctcatcctaagcgcagctct-3',
 5'-gggggcataactctcttgg-3'

The amplification reaction was 50°C for 2 minutes and 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minutes, and terminated by a cooling step at 4°C. The mean C_T derived from group C was used as the calibrator. The equation used was as follows:

$$\text{Ratio (sample to calibrator)} = 2^{(-\Delta\Delta C_T)}$$

where $\Delta C_T = C_T(\text{Goal Gene}) - C_T(\text{Reference gene})$, and $\Delta\Delta C_T = \Delta C_T(\text{sample}) - \Delta C_T(\text{calibrator})$.

Statistical Methods

All values were expressed as the mean $\pm$ SD. SPSS version 13.0 software (SPSS, Inc, an IBM Company, Chicago, Illinois) was used for statistical analysis. Both repeated measurements and analysis of variance (ANOVA) were used in the significance test of differences among groups. The least significant difference (LSD) method was applied in pairwise comparisons when variance was homogeneous. The Tamhane T2 method was used when variance was heterogeneous.

Results

NF- κ Bp65 protein was detected by immunohistochemistry in the nasal mucosa. As seen in **Figure 1**, NF- κ Bp65 protein in group A was only expressed in the cytoplasm, whereas it was expressed in both cytoplasm and nucleus in groups B, C1, C2, and C3. From **Table 1**, the MOD of NF- κ Bp65 in group B was increased compared with group A ($P = .003$). After AR rats were treated with PDTC, the MOD of NF- κ Bp65 in groups C1, C2, and C3 was still significantly higher than in group A ($P = .004$, $P = .005$, and

$P = .007$, respectively). Compared with group B, the MOD of NF- κ Bp65 in groups C1, C2, and C3 was significantly reduced ($P = .041$, $P = .038$, and $P = .005$, respectively). However, in group C2, expression was reduced compared with group C1 ($P = .041$), and in group C3, expression was significantly lower than in C1 and C2 ($P = .009$ and $P = .013$).

NF- κ Bp65 protein was detected by Western blotting of the nuclear fraction from nasal mucosa (**Figure 2**). As seen in **Table 1**, NF- κ Bp65 protein in group A was not detected by Western blotting, but significant NF- κ Bp65 was detected in groups B, C1, C2, and C3. The value of NF- κ Bp65 in group B was higher than in groups C1, C2, and C3 ($P = .050$, $P = .018$, and $P = .009$, respectively). The value in group C2 was lower compared with group C1 ($P = .039$), whereas the value in group C3 was lower compared with groups C1 and C2 ($P = .028$ and $P = .012$, respectively).

Immunohistochemistry results show MUC5AC was mostly localized in the cell cytoplasm in the nasal mucosa (**Figure 3**). As seen in **Table 1**, the MOD of the positive areas of MUC5AC in groups B, C1, C2, and C3 was higher than in group A ($P = .004$, $P = .005$, $P = .008$, and $P = .021$, respectively). After treatment with PDTC, the MOD of MUC5AC in groups C1, C2, and C3 decreased significantly compared with that in group B ($P = .039$, $P = .009$, and $P = .006$, respectively). The MOD of MUC5AC in group C2 was lower than in group C1 ($P = .043$), whereas the expression in group C3 was lower compared with groups C1 and C2 ($P = .019$ and $P = .045$, respectively).

The MUC5AC protein in the nasal mucosa was detected by Western blotting and expressed as a ratio to the β -actin expression in the same sample (**Figure 4**). From **Table 1**, we see that the MUC5AC level in groups B, C1, C2, and C3 was significantly increased compared with group A ($P = .007$, $P = .008$, $P = .009$, and $P = .035$, respectively). After AR rats were treated with PDTC, the MUC5AC level in treated groups C1, C2, and C3 was reduced compared with group B ($P = .046$, $P = .038$, and $P = .009$, respectively). The expression in group C2 was lower compared with group C1 ($P = .049$), and in group C3, it was lower compared with groups C1 and C2 ($P = .030$ and $P = .048$, respectively).

From **Table 1**, the level of mRNA coding for MUC5AC in groups B, C1, C2, and C3 was significantly higher than in group A ($P = .007$, $P = .007$, $P = .008$, and $P = .009$, respectively). After the treatment with PDTC, the mRNA for MUC5AC in treated groups C1, C2, and C3 was significantly reduced ($P = .041$, $P = .035$, and $P = .008$, respectively) compared with group B, whereas expression in group C2 was lower compared with group C1 ($P = .015$), and mRNA in group C3 was significantly lower than in groups C1 and C2 ($P = .017$ and $P = .011$, respectively).

From **Table 2**, we can see that the level of mRNA coding for TNF- α in the nasal mucosa in groups B, C1, C2, and C3 was significantly increased compared with group A ($P = .002$, $P = .003$, $P = .005$, and $P = .008$, respectively). After the AR rats were treated with PDTC, the mRNA for

Table 1. Expression of NF- κ Bp65 and MUC5AC by Immunohistochemistry, Western Blotting, and Real-time PCR

	NF- κ Bp65			MUC5AC		
	Immunohistochemistry		Western Blotting		Western Blotting	
	Mean $\pm$ SD	P Value	Mean $\pm$ SD	P Value	Mean $\pm$ SD	P Value
Group A	0.011 $\pm$ 0.0013		0		0.312 $\pm$ 0.0216	
Group B	0.130 $\pm$ 0.0065	.003 ^a	0.602 $\pm$ 0.0908	.000 ^a	0.834 $\pm$ 0.0531	.007 ^a
Group C1	0.115 $\pm$ 0.0024	.004 ^a	0.571 $\pm$ 0.0797	.000 ^a	0.703 $\pm$ 0.0350	.008 ^a
		.041 ^b		.050 ^b		.046 ^b
Group C2	0.100 $\pm$ 0.0065	.005 ^a	0.386 $\pm$ 0.0709	.000 ^a	0.621 $\pm$ 0.0381	.009 ^a
		.038 ^b		.018 ^b		.038 ^b
		.041 ^c		.039 ^c		.049 ^c
Group C3	0.063 $\pm$ 0.0021	.007 ^a	0.104 $\pm$ 0.0449	.000 ^a	0.524 $\pm$ 0.0160	.035 ^a
		.005 ^b		.009 ^b		.009 ^b
		.009 ^c		.028 ^c		.030 ^c
		.013 ^d		.012 ^d		.048 ^d
n = 5 in each group. Abbreviations: NF, nuclear factor; PCR, polymerase chain reaction. ^a Versus group A. ^b Versus group B. ^c Versus group C1. ^d Versus group C2.						

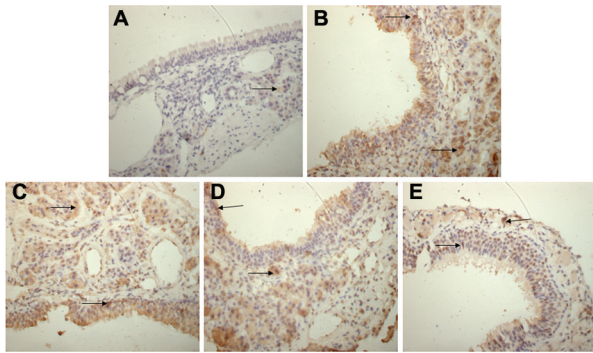


Figure 1. The location and positive distribution of NF-κBp65 in rat nasal mucosa by immunocytochemistry. A, B, C, D, and E represent group A, group B, group C1, group C2, and group C3, respectively. Arrows represent the positive expression of NF-κBp65 in rat nasal mucosa.

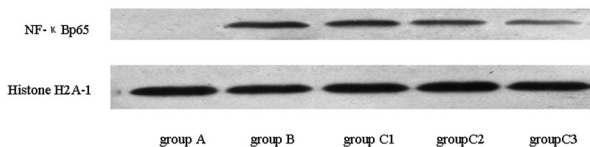


Figure 2. Western blotting for NF-κBp65 in rat nasal mucosa. Histone H2A-I was used as loading control. The intensity of NF-κBp65 bands was normalized to the intensity of histone H2A-I bands.

TNF-α in treated groups C1, C2, and C3 was significantly reduced compared with group B ($P = .018$, $P = .008$, and $P = .005$, respectively). The expression in group C2 was lower compared with group C1 ($P = .015$), and in group C3, it was lower compared with groups C1 and C2 ($P = .007$ and $P = .011$, respectively). In the nasal mucosa, the level of mRNA that was coding for IL-6 in groups B, C1, C2, and C3 was significantly increased compared with group A ($P = .003$, $P = .005$, $P = .006$, and $P = .009$, respectively). After treatment with PDTC, IL-6 mRNA in groups C1, C2, and C3 was reduced compared with group B ($P = .026$, $P = .009$, and $P = .006$, respectively). Expression in group C2 was reduced compared with group C1 ($P = .021$), and expression in group C3 was lower compared with groups C1 and C2 ($P = .009$ and $P = .012$, respectively).

From **Table 2**, we can see that the level of TNF-α in the NLF in groups B, C1, C2, and C3 was decreased significantly compared with group A ($P = .005$, $P = 0.005$, $P = .006$, and $P = .007$, respectively). After the treatment with PDTC, the level of TNF-α in groups C1, C2, and C3 was significantly reduced compared with group B ($P = .018$, $P = .008$, and $P = .005$, respectively). Tumor necrosis factor-α expression in group C2 was lower compared with group C1 ($P = .039$), and in group C3, it was reduced compared with both groups C1 and C2 ($P = .017$ and $P = .011$, respectively). Similarly, in the NLF (**Table 2**), the level of IL-6 in groups B, C1, C2, and C3 was

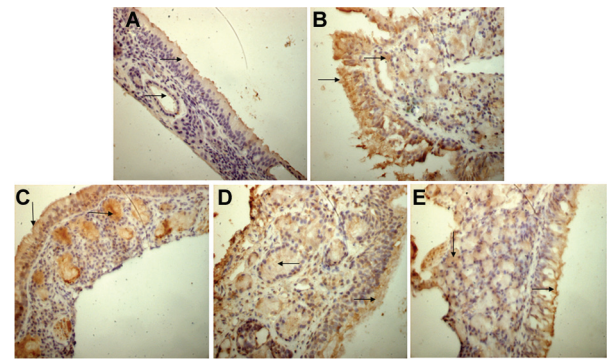


Figure 3. The location and positive distribution of MUC5AC in rat nasal mucosa by immunocytochemistry. A, B, C, D, and E represent group A, group B, group C1, group C2, and group C3, respectively. Arrows represent the positive expression of MUC5AC in rat nasal mucosa.

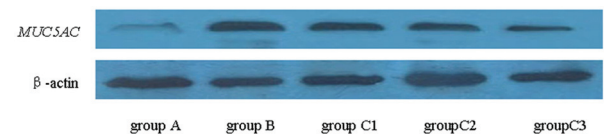


Figure 4. Western blotting for MUC5AC in rat nasal mucosa. β-Actin was used as loading control. The intensity of MUC5AC bands was normalized to the intensity of β-actin bands.

significantly increased compared with the level in group A ($P = .006$, $P = .006$, $P = .007$, and $P = .008$, respectively). After the treatment with PDTC, the level of IL-6 mRNA in groups C1, C2, and C3 was reduced compared with group B ($P = .041$, $P = .035$, and $P = .008$, respectively). In group C2, expression was lower compared with group C1 ($P = .039$), and the mRNA level in group C3 was reduced compared with groups C1 and C2 ($P = .017$ and $P = .011$, respectively).

Discussion

Recent studies show that MUC5AC is a major mucin secreted during airway pathology. Various inflammatory mediators, pathogenic microorganisms, and their products can activate transcription factors and bind them to the promoter elements of the MUC5AC gene through a variety of signaling pathways, causing the increase of mucin synthesis.¹³ The effective control of hypersecretion and understanding the mechanism of secretion are focuses of AR research.

NF-κB Activation Induced the Transcription of Inflammatory Cytokines TNF-α and IL-6 in AR

A successful animal model of AR was achieved using ovalbumin with Al(OH)₃ as an adjuvant.¹⁴⁻¹⁶ Allergic inflammatory responses are orchestrated by several transcription factors, particularly NF-κB.¹⁷ Our study shows that NF-κBp65 protein in group A was only expressed in the

Table 2. Expression of IL-6 and TNF- α by Real-time PCR or ELISA

	TNF- α			IL-6		
	Real-time PCR		P Value	Real-time PCR		P Value
	Mean $\pm$ SD	ELISA, ng/L		Mean $\pm$ SD	ELISA, ng/L	
Group A	I 19.47 $\pm$ 1.36	3.591 $\pm$ 0.9551		I 14.36 $\pm$ 1.42	9.267 $\pm$ 0.7998	
Group B		85.625 $\pm$ 2.8268	.005 ^a		62.918 $\pm$ 1.8327	.006 ^a
Group C1	17.38 $\pm$ 1.01	80.481 $\pm$ 2.9638	.003 ^a .018 ^b	13.15 $\pm$ 1.05	60.353 $\pm$ 1.3656	.005 ^a .026 ^b
Group C2	13.12 $\pm$ 1.34	76.802 $\pm$ 2.2322	.005 ^a .008 ^b	10.17 $\pm$ 0.36	56.746 $\pm$ 1.8601	.006 ^a .009 ^b
Group C3	9.74 $\pm$ 2.12	65.747 $\pm$ 3.2917	.015 ^c .008 ^a .005 ^b .007 ^c .011 ^d	6.18 $\pm$ 1.34	49.505 $\pm$ 3.1610	.021 ^c .009 ^a .006 ^b .009 ^c .012 ^d

n = 5 in each group. Abbreviations: ELISA, enzyme-linked immunosorbent assay; IL-6, interleukin-6; PCR, polymerase chain reaction; TNF- α , tumor necrosis factor- α .

^aVersus group A.

^bVersus group B.

^cVersus group C1.

^dVersus group C2.

cytoplasm and not in the nucleus. A significant amount of NF- κ Bp65 in group B was seen in the nucleus by immunohistochemistry and Western blotting, indicating that the NF- κ B was activated in AR. It had been elsewhere reported that NF- κ Bp65 is activated in AR, and the amount of NF- κ Bp65 in the nucleus is increased.¹

In our results, the mRNA for TNF- α and IL-6 in group B and the levels in the NLF were increased in AR rats. When we treated AR rats with PDTC, we found that the nuclear localization of NF- κ Bp65 was decreased in a time-dependent manner, indicating PDTC inhibited NF- κ B activation. At the same time, TNF- α or IL-6 mRNA and cytokine secretion were also decreased. Nuclear localization of NF- κ Bp65 induces gene transcriptions¹⁸ and regulates a wide variety of cellular genes, including several cytokines.¹⁹ NF- κ B activates gene transcription and secretion of an array of inflammatory cytokines, including TNF- α and IL-6, and the cytokines can further activate the NF- κ B pathway as extracellular factors. NF- κ B plays an important role in the regulation of AR inflammatory cytokine networks.^{20,21} We concluded that NF- κ B activation upregulated inflammatory cytokine production.

NF- κ B Induced *MUC5AC* Hypersecretion through the Upregulation of Inflammatory Cytokines TNF- α and IL-6 in AR

Numerous reports have shown that inflammatory cytokines can stimulate *MUC5AC* gene expression in airway epithelial cells in vitro and in vivo.²² Tumor necrosis factor- α and IL-6 have been implicated as key regulators of *MUC5AC* gene expression. Tumor necrosis factor- α has been shown to regulate *MUC5AC* expression at the transcriptional level in human airway epithelial cells.²³ Likewise, IL-6 has also been shown to increase the steady-state expression of *MUC5AC* mRNA in differentiated cultures of primary human tracheobronchial epithelial cells.²⁴ Kim et al²⁵ demonstrated the involvement of NF- κ B in *MUC5AC* upregulation by IL-6 in an asthma model in C57BL/6 mice. Wang et al²⁶ observed that exposure of conjunctival epithelial cells transfected with *MUC5AC*-luciferase plasmids to TNF- α and IL-6 led to increased *MUC5AC* gene expression and confirmed that *MUC5AC* gene expression is regulated by proinflammatory cytokines. Smirnova et al²⁷ found that exposure of goblet cells to IL-6 and TNF- α significantly increased *MUC5AC* secretion in a time and dose-dependent manner. It has been reported that TNF- α induces *MUC5AC* overexpression in human nasal polyp epithelial cells.²⁸ However, it remains to be determined whether an NF- κ B-based mechanism is involved in cytokine-stimulated mucin upregulation.

In this experiment, we found that *MUC5AC* protein in the AR model group was significantly increased, whereas the production of *MUC5AC* protein was reduced in a time-dependent manner after inhibiting NF- κ B activation. The changes in transcription from the *MUC5AC* gene were consistent with changes of TNF- α and IL-6 during the experiment,

indicating that *MUC5AC* expression might be upregulated by TNF- α and IL-6.

In addition, it has been reported that the p38 MAPK pathway is involved in the upregulation of *MUC5AC* in AR.²⁹ Inflammatory cytokines TNF- α and IL-1 β can induce *MUC5AC* overexpression through a mechanism involving p38 MAPK activation in human airway epithelial cells.²³ In this study, NF- κ B activation induced the transcription and secretion of the inflammatory cytokine TNF- α in the AR rats, and the NF- κ B inhibitor inhibited this pathway. Therefore, the NF- κ B inhibitor might also affect p38 MAPK activation by inhibiting the transcription and secretion of the inflammatory cytokines TNF- α and IL-6, which downregulated *MUC5AC* expression in AR rats.

Conclusion

NF- κ B was activated in AR, resulting in the nuclear localization of NF- κ Bp65. NF- κ Bp65 upregulates the transcription and secretion of inflammatory cytokines TNF- α and IL-6, resulting in the increase in *MUC5AC* production. These factors led to the hypersecretion from the nasal mucosa of AR rats. We speculate that NF- κ B upregulation of the transcription of TNF- α and IL-6 induces *MUC5AC* overexpression.

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Author Contributions

Weiwei Wang, in charge of the detection of real-time PCR, statistics of experimental data, and the writing of the manuscript; **Ming Zheng**, in charge of the detection of immunohistochemistry, Western blotting, and ELISA.

Disclosures

Competing interests: None.

Sponsorships: None.

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