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Effect of Asian Sand Dust on Mucin Production in NCI-H292 Cells and Allergic Murine Model

Joo Hyun Jung, MD¹, Il Gyu Kang, MD¹, Heung Eog Cha, MD¹,
Sung Ho Choe, MD¹, and Seon Tae Kim, MD¹

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Abstract

Objective. To investigate the effect of Asian sand dust (ASD) on mucin production in human respiratory epithelial cells in vitro and in allergic murine nasal epithelial cells.

Study Design. Controlled, in vitro.

Setting. Academic research laboratory.

Materials and Methods. Human NCI-H292 cells were treated with ASD and analyzed by immunostaining, reverse transcriptase–polymerase chain reaction for MUC5AC mRNA expression, and Periodic Acid Schiff (PAS) staining. Forty female BALB/c mice were classified into 4 groups. Two groups were sensitized with ovalbumin (OVA), and 1 of these was treated with ASD (ASD+OVA). The 2 nonsensitized groups were treated with ASD or saline. Then the murine nasal mucosal tissues were evaluated by hematoxylin and eosin (H&E) staining, PAS staining, and immunostaining for MUC5AC and transforming growth factor (TGF)- α proteins.

Results. The numbers of MUC5AC-immunopositive NCI-H292 cells and PAS-positive NCI-H292 cells were significantly higher in the ASD-treated cells than in the control cells ($P = .039$ and $P = .029$, respectively). MUC5AC mRNA expression in the cells increased with increasing concentrations of ASD. In the murine nasal epithelial tissues, the numbers of eosinophils and PAS-positive cells were significantly higher in the ASD+OVA group than in the OVA group (H&E staining, $P = .037$; PAS staining, $P = .019$). At 2 weeks, the numbers of MUC5AC- and TGF- α -positive cells in the nasal epithelial tissue were significantly higher in the ASD+OVA group than in the OVA group ($P = .031$ and $P = .033$, respectively).

Conclusion. ASD can induce mucin production in respiratory epithelial cells.

Keywords

Asian sand dust, mucin production, NCI-H 292 cells, allergic mouse

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Asian sand dust (ASD) is a natural phenomenon originating from the deserts of China and Mongolia. It has caused extensive damage to the social economies of Korea, Japan, and North America.¹ ASD is composed of many pollutants, especially particulate matter less than 10 μm in diameter (PM10), which represents 53% to 71% of ASD. PM10 can reach the lower respiratory tract, causing respiratory tract disease.^{2–4} Recent epidemiological studies have shown that ASD events are associated with symptom aggravation in asthmatic patients and increased daily mortality in South Korea.^{5,6} Lei et al⁷ found increased numbers of white blood cells in the serum and increased inflammatory cells in the bronchoalveolar lavage fluid of mice exposed to ASD, suggesting that ASD can cause pulmonary inflammation and lung damage.

Although many studies have reported that ASD influences allergic inflammation in mice, few studies have focused on mucus production induced by ASD.^{8,9} Mucus plays a crucial role in protecting the airways by capturing bacteria and particulate matter and by maintaining the humidity of the airway. Mucus hypersecretion aggravates airway obstruction in many airway inflammatory diseases.^{10–13} The present study was conducted to investigate the effect of ASD on mucus production in vitro in human NCI-H292 cells and in nasal epithelial cells from an allergic murine model.

Materials and Methods

Study Protocol

This study was approved by the Institutional Review Board of Gachon Medical School, Gil Medical Center, Incheon, Korea.

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Preparation of Particulates

ASD was collected from air dust at 500 L/min for 6 hours per day using a high-volume sampler (HV500F; Sibata, Soka, Japan) with a PM2.5 filter pack, during ASD warnings issued by the Korean Meteorological Association. The dust-covered filter paper was removed from the air sampler and cut into pieces, which were placed in a 10-mL tube containing phosphate-buffered saline (PBS). The tube contents were mixed using a vortexer, the pieces of wet filter paper were removed, and the remaining solution was passed through a filter with 10- μ m pores to collect PM10. The PM10 was weighed, suspended by sonication (5×30 seconds at 3–5 W), and distributed into 1.5-mL tubes. The samples were sterilized in an autoclave at 121°C for 15 minutes and stored at –20°C.

Analysis of ASD Components

The concentration of each element in ASD was determined by inductively coupled plasma optical emission spectrometry (Optima 5300 DV; Perkin-Elmer, Waltham, Massachusetts) and inductively coupled plasma mass spectrometry (Elan DRE II; Perkin-Elmer). The concentration of endotoxin was determined using a quantitative chromogenic Limulus amoebocyte lysate assay (QCL-1000; Lonza, Walkersville, Maryland) following the manufacturer's instructions.

Cell Culture and Stimulation

NCI-H292 human pulmonary mucoepidermoid carcinoma cells (ATCC, Manassas, Virginia) were plated at 1×10^5 to 2×10^5 cells per well in 8-well plates and incubated in RPMI-1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μ g/mL streptomycin, and 25 mM HEPES at 37°C in an atmosphere of 5% CO₂. After 2 to 3 days, the cells reached 90% confluence and were incubated in serum-free medium for 24 hours. The serum-free medium was removed, the cells were washed, and 2 mL of RPMI medium containing the prepared PM10 (hereafter referred to as ASD) at a concentration of 0, 10, 100, 250, or 500 μ g/mL were added to respective wells. After the cells were incubated for 24 hours, the supernatant was collected. As a positive control, phorbol 12-myristate 13-acetate (PMA; 50 ng/mL) was added to the serum-free medium for 1 hour prior to incubation. As a negative control, only serum-free medium was added.

Reverse Transcriptase–Polymerase Chain Reaction

Total RNA was extracted using TRIzol reagent (Gibco, Grand Island, New York) according to the manufacturer's instructions. For cDNA synthesis, total RNA (2 μ g), Maloney murine leukemia virus reverse transcriptase (Life Technologies, Gaithersburg, Maryland), 5 $\times$ reverse transcription buffer, 10 mM dNTPs, RNase inhibitor, and random hexamer primer in a final volume of 20 μ L were incubated at 37°C for 1 hour. PCR amplification was performed in a 50- μ L reaction volume containing cDNA, 10 $\times$ PCR reaction buffer, 25 mM MgCl₂, 2.5 mM dNTPs, and a *MUC5AC* primer set

(forward, 5'-TCCGGCCTCATCTTCTCC-3'; reverse, 5'-ACTTGGGCACTGGTGCTG-3') or a *GAPDH* primer set (forward, 5'-GTGGATATTGTTGCCATCAATGACC-3'; reverse, 5'-GCCCCAGCCTTCATGGTGGT-3'). The PCR conditions were denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 45 minutes, 55°C for 30 minutes, and 72°C for 1 minute, with a final extension at 72°C for 7 minutes. The PCR products (10 μ L) were analyzed on 2% agarose gels stained with ethidium bromide.

MTT Assay

Cell viability was assessed by the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) assay. NCI-H292 cells were cultured with various concentrations of the prepared ASD and washed with PBS. Then, 100 μ L of MTT solution (5 mg/mL) were added to each well, and the cells were incubated at 37°C for 4 hours. The MTT solution was removed, 100 μ L of dimethyl sulfoxide was added to each well, and the plate was incubated at 37°C for 15 minutes. The absorbance at 492 nm was measured with a spectrometer. All experiments were repeated at least 3 times.

Allergic Murine Model

Forty female BALB/c mice (6 weeks of age) were purchased (Central Lab, Animal Inc, Seoul, Korea), and all mice were checked for infections. This study adhered to the Gachon University Animal Research guidelines. The mice were divided equally among 4 groups: saline, ASD, ovalbumin (OVA), and ASD+OVA groups ($n = 10$ in each group). The mice in the saline group were exposed to 1 mL of normal saline via a nebulizer (Omron, Tokyo, Japan) for 15 minutes each day for 7 days. The mice in the ASD group were exposed to 10 mg of ASD in 1 mL of normal saline via a nebulizer for 15 minutes each day for 7 days.

The other 2 groups were used to develop the allergic murine model. To induce systemic sensitization, the abdominal cavity of each mouse was injected with 300 μ L of normal saline containing 25 μ g of OVA (Sigma-Aldrich, St Louis, Missouri) and 1 mg of Al(OH)₃ gel (Alum; Pierce Chemical Co, Rockford, Illinois) on days 1, 7, and 14. The systemically sensitized mice were locally exposed to 5 mL of 2% OVA for 30 min 4 times per day, starting on day 21. Local sensitization was performed in an acrylic box (40 $\times$ 40 $\times$ 30) connected to the nebulizer through a hole in its side (**Figure 1**).

ASD Stimulation in an Allergic Murine Model

The sensitized mice were divided into the OVA ($n = 10$) and OVA+ASD ($n = 10$) groups. The OVA group of sensitized mice were exposed to 5 mL of 2% OVA for 30 minutes once every other day to sustain the sensitized state. The mice in the OVA+ASD group were exposed to 5 mL of 2% OVA plus 1 mL of normal saline containing 10 mg of ASD for 30 minutes per day for 1 or 2 weeks.

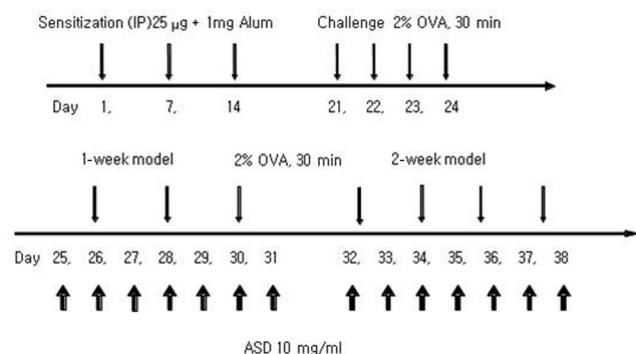


Figure 1. Allergic mouse model.

Extraction of Nasal Specimen

On the day of sacrifice, the head of each mouse was harvested and fixed in 10% paraformaldehyde for 24 hours. The specimens were decalcified in a decalcifying reagent (Decalcified II; Surgipath Medical Industries Inc, Richmond, Illinois) for 5 days and sectioned from behind the upper incisor to 2 mm in front of the incisive papilla of the hard palate. The sections were embedded in paraffin, and the paraffin block was cut at intervals of 4 µm.

Hematoxylin and Eosin Staining

To determine the degree of inflammation, nasal mucosal specimens were stained with hematoxylin for 45 seconds, washed, and then stained with eosin for 20 seconds.

Periodic Acid Schiff staining

To evaluate the production of mucus glycoproteins, NCI-H292 cells or nasal mucosal specimens were incubated with periodic acid for 5 minutes at 4°C, washed with distilled water, and incubated with Schiff's reagent (Sigma-Aldrich) for 15 minutes, followed by staining with hematoxylin for 3 minutes. The average number of Periodic Acid Schiff (PAS)-positive NCI-H292 cells was determined after counting 10 different fields under a microscope at 200× magnification. The ratio of PAS-positive cells to total epithelial cells in the nasal mucosal epithelial layer was determined under a microscope at 100× magnification.

Immunohistochemical Staining

NCI-H292 cells or nasal specimens were incubated with monoclonal antibodies against human MUC5AC (Neomarker Co, Fremont, California) and transforming growth factor (TGF)-α (Abcam, Cambridge, UK) diluted 1:200 for 90 minutes at room temperature or overnight at 4°C, respectively, followed by incubation with goat antimouse immunoglobulin (Santa Cruz Biotechnology, Santa Cruz, California) for 1 hour at room temperature. Immunoreactive proteins were visualized by incubation with streptavidin-conjugated peroxidase and 3,3'-diaminobenzidine tetrahydrochloride. Sections were counterstained with hematoxylin and eosin (H&E). The primary antibody was omitted for the controls. The average

Table 1. Chemical Analysis of Asian Sand Dust Particles Determined by Inductively Coupled Plasma Optical Emission Spectrometry and Inductively Coupled Plasma Mass Spectrometry

Component	Chemical Contents Fraction, %
SiO ₂	52.3
Al ₂ O ₃	16.0
Fe ₂ O ₃	5.92
CaO	4.31
K ₂ O	2.51
MgO	2.48
Na ₂ O	1.68
TiO ₂	0.73
P ₂ O ₅	0.15
MnO	0.12
ZnO	0.06
BaO	0.05
SrO	0.02
Other trace elements ^a	0.21
Loss on ignition	13.46

^aThe elements that showed amounts of <0.01% (Cu, Cr, Pb, As, Li, Be, V, Ni, Co, Ga, Se, Rb, Cd, Hg, In, Cs, Bi, U, Sb, Au, Hf, Ir, Pd, Sn, Mo, Nb, Re, Ta, W, Ce, Dy, Eu, Er, Gd, Ho, La, Lu, Nd, Pr, Sm, Sc, Tb, Th, Tm, Yb, Y, B, Rh, Ge).

number of immunopositive cells among total NCI-H292 cells was calculated after counting 10 different fields under a microscope at 200× magnification. The ratio of immunopositive cells to total epithelial cells in the nasal mucosal epithelial layer was determined under a microscope at 100× magnification.

Statistical Analysis

The differences between 2 samples of NCI-H292 cells were analyzed using the Mann-Whitney *U* test. Pathological results were analyzed using the Kruskal-Wallis test. All analyses were performed using SPSS for Windows, version 10.0 (SPSS Inc, Chicago, Illinois). Values of *P* < .05 indicated significance.

Results

Characteristics of ASD

The most abundant component of ASD was SiO₂ (52.3%). Other major components included Al₂O₃ (16.0%), Fe₂O₃ (5.92%), CaO (4.31%), K₂O (2.51%), MgO (2.48%), and Na₂O (1.68%). Many minor and trace elements were present at less than 0.1%. No endotoxin was detected in the autoclaved ASD samples (Table 1).¹⁴

NCI-H 292 Cells

Immunohistochemical staining. NCI-H292 cells were treated with PMA (positive control) or increasing concentrations of ASD and were analyzed by cell staining, reverse transcriptase-polymerase chain reaction (RT-PCR), and MTT assay. The number of MUC5AC-positive NCI-H292 cells was significantly higher among the PMA-treated and

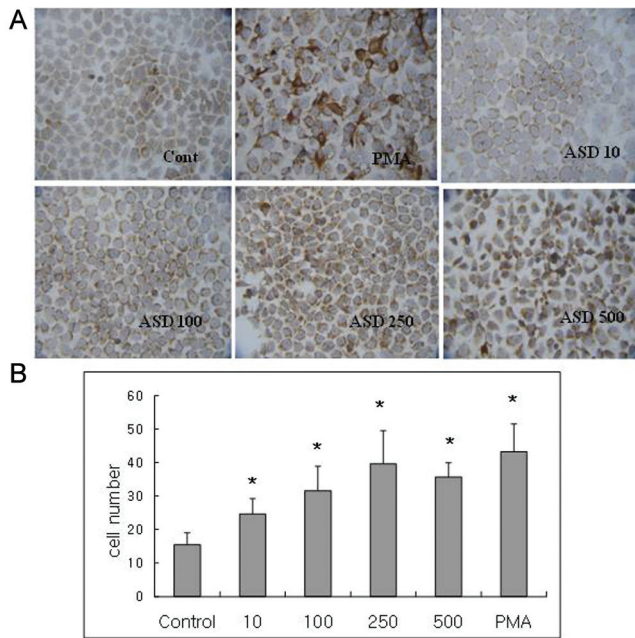


Figure 2. Immunohistochemical staining of NCI-H292 cells. The number of MUC5AC-positive cells increased with increasing concentrations of Asian sand dust (ASD). * $P < .05$ ($P = .043$ in ASD 10, $P = .037$ in ASD 100, $P = .029$ in ASD 250, $P = .039$ in ASD 500, $P = .030$ in PMA).

ASD-treated cells than among the control cells (for control vs treatment with 10 $\mu\text{g/mL}$ of ASD [ASD 10], $P = .043$; vs ASD 100, $P = .037$; vs ASD 250, $P = .029$; vs ASD 500, $P = .039$; vs PMA, $P = .030$; **Figure 2**).

PAS staining. The number of PAS-positive cells was significantly higher among the cells treated with PMA and cells treated with ASD at concentrations of 250 $\mu\text{g/mL}$ ($36.08\% \pm 7.23\%$) and higher than among the control cells ($14.75\% \pm 4.51\%$; control vs ASD 250, $P = .038$; vs ASD 500, $P = .029$; vs PMA, $P = .030$; **Figure 3**).

RT-PCR. The expression of *MUC5AC* mRNA (679 bp) relative to that of *GAPDH* mRNA in NCI-H292 cells increased with increasing concentrations of ASD, from 10 to 500 $\mu\text{g/mL}$ (**Figure 4**).

MTT assay. The MTT cell viability assay revealed fewer viable NCI-H292 cells at 500 $\mu\text{g/mL}$ ASD (**Figure 5**).

Results in an Allergic Murine Model

H&E staining. In the allergic murine model mice, the number of eosinophils in the nasal mucosal tissue was significantly higher in the OVA+ASD group ($8.54\% \pm 1.72\%$) than in the OVA group ($3.53\% \pm 1.10\%$; $P = .032$) at 1 week after exposure to ASD. At 1 and 2 weeks postexposure to ASD, the number of eosinophils was significantly higher in the OVA+ASD group compared with the saline ($P = .031$ and $P = .023$, respectively), ASD ($P = .032$ and $P = .024$, respectively), and OVA groups ($P = .046$ and $P = .037$, respectively). None of the groups showed a significant change in the number of eosinophils between weeks 1 and 2 (**Figure 6**).

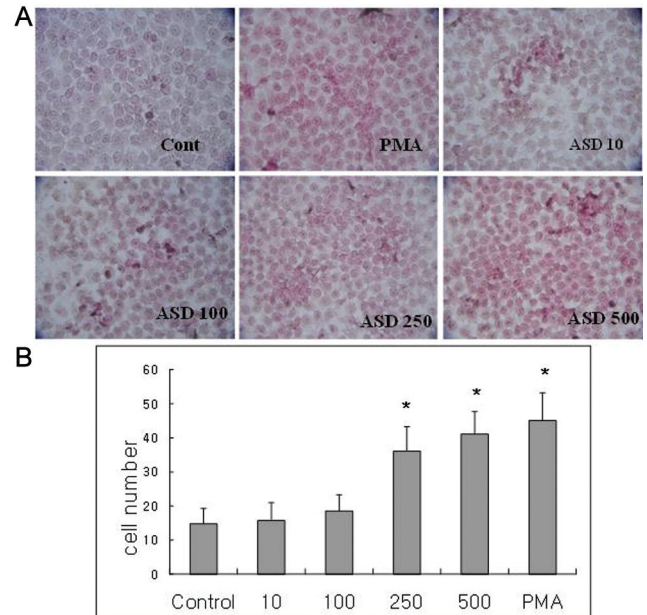


Figure 3. Periodic Acid Schiff (PAS) staining of NCI-H292 cells. The number of PAS-positive NCI-H292 cells increased with increasing concentrations of Asian sand dust (ASD). * $P < .05$ ($P = .038$ in ASD 250, $P = .029$ in ASD 500, $P = .030$ in PMA).

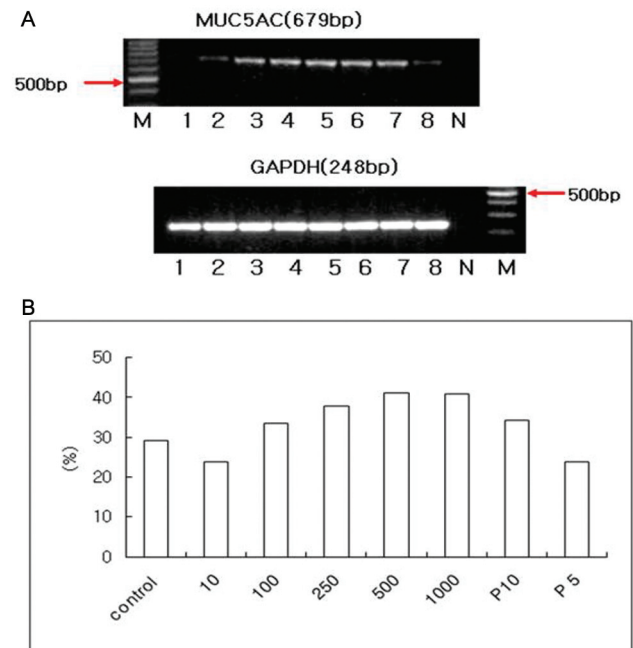


Figure 4. Reverse transcriptase–polymerase chain reaction analysis of NCI-H292 cells. The expression of *MUC5AC* mRNA (679 bp) in NCI-H292 cells increased with increasing concentrations of Asian sand dust, from 10 to 500 $\mu\text{g/mL}$.

PAS staining. Similarly, the number of PAS-positive cells was significantly higher in the OVA+ASD group ($60.37\% \pm 3.12\%$) than in the OVA group ($34.54\% \pm 3.88\%$; $P = .009$).

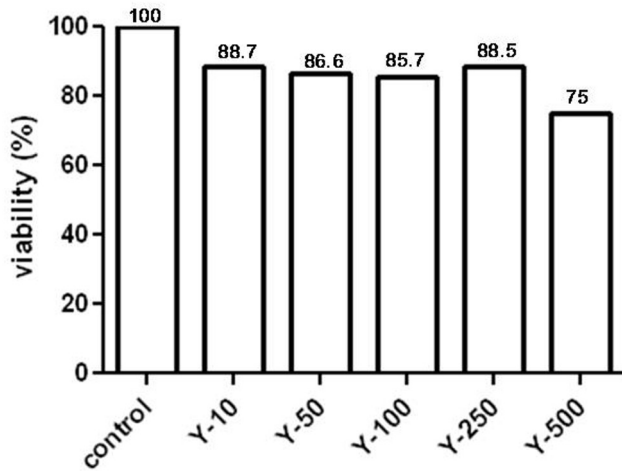


Figure 5. MTT assay of NCI-H292 cell viability. NCI-H292 cell viability decreased after exposure to 500 $\mu\text{g/mL}$ Asian sand dust (ASD). Y-10: ASD 10 $\mu\text{g/mL}$, Y-50: ASD 50 $\mu\text{g/mL}$, Y-100: ASD 100 $\mu\text{g/mL}$, Y-250: ASD 250 $\mu\text{g/mL}$, Y-500: ASD 500 $\mu\text{g/mL}$.

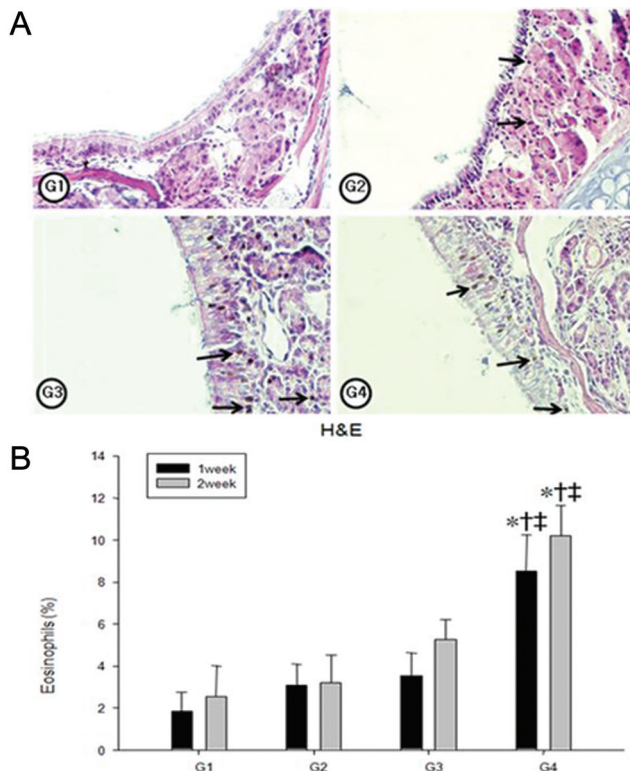


Figure 6. Hematoxylin and eosin staining of nasal mucosal tissue from mice. The number of eosinophils in the epithelial layer and submucosa was significantly increased in OVA+ASD-treated mice compared with saline-, ASD-, or OVA-treated mice (magnification, 100 $\times$). $\rightarrow$: eosinophil. G1: control, G2: ASD, G3: OVA, G4: OVA+ASD. * $P < .05$ versus the G1 ($P = .031$ in the first week, $P = .023$ in the second week), $\dagger P < .05$ versus the G2 ($P = .032$ in the first week, $P = .024$ in the second week), $\ddagger P < .05$ versus the G3 ($P = .046$ in the first week, $P = .037$ in the second week). % = eosinophils/total cells $\times 100$. ASD indicates Asian sand dust; OVA, ovalbumin.

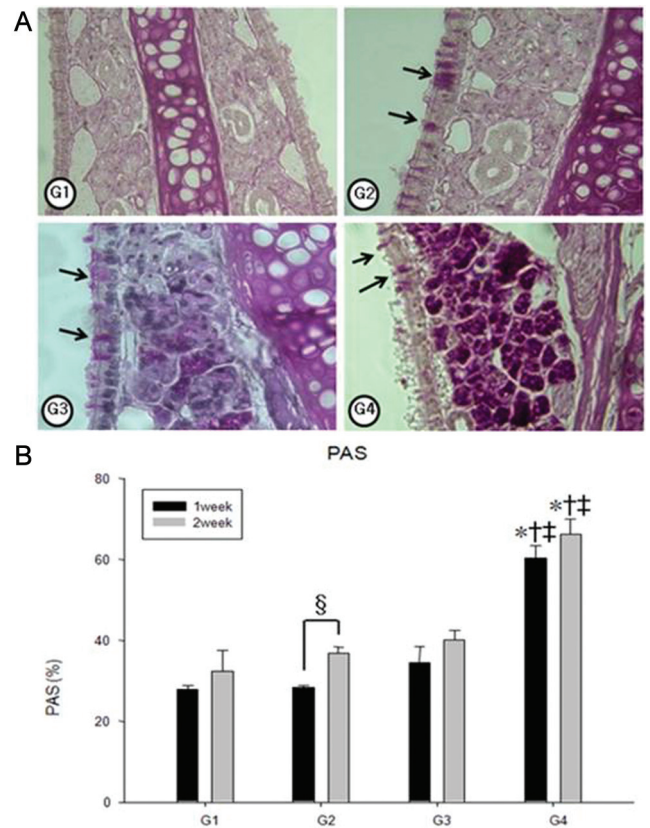


Figure 7. Periodic Acid Schiff (PAS) staining of nasal mucosal tissue from mice. The number of PAS-positive cells in the epithelial layer and submucosa was significantly increased in OVA+ASD-treated mice compared with saline-, ASD-, or OVA-treated mice (magnification, 100 $\times$). $\rightarrow$: positive cell. G1: control, G2: ASD, G3: OVA, G4: OVA+ASD. * $P < .05$ versus the G1 ($P = .027$ in the first week, $P = .031$ in the second week), $\dagger P < .05$ versus the G2 ($P = .022$ in the first week, $P = .034$ in the second week), $\ddagger P < .05$ versus the G3 ($P = .009$ in the first week, $P = .019$ in the second week), $\S P = .032$ 1-week period versus 2-week period. % = stained epithelial cells/total epithelial cells $\times 100$. ASD indicates Asian sand dust; OVA, ovalbumin.

at 1 week after ASD exposure, and the number of PAS-positive cells was significantly higher in the OVA+ASD group than in the saline ($P = .027$ and $P = .031$, respectively), ASD ($P = .022$ and $P = .034$, respectively), and OVA groups ($P = .009$ and $P = .019$, respectively) at 1 and 2 weeks postexposure (**Figure 7**).

MUC5AC. The number of MUC5AC-immunopositive cells was significantly higher in the OVA+ASD group ($63.65\% \pm 5.32\%$) than in the OVA group ($37.56\% \pm 0.77\%$; $P = .031$) at 2 weeks after exposure to ASD. The number of MUC5AC-immunopositive cells was significantly higher at 2 weeks than at 1 week postexposure in both the OVA+ASD and OVA groups ($P = .020$ and $P = .033$, respectively; **Figure 8**).

TGF- α . At 2 weeks postexposure to ASD, the number of TGF- α -immunopositive cells was significantly higher in the OVA+ASD group ($34.58\% \pm 4.53\%$) than in the OVA group

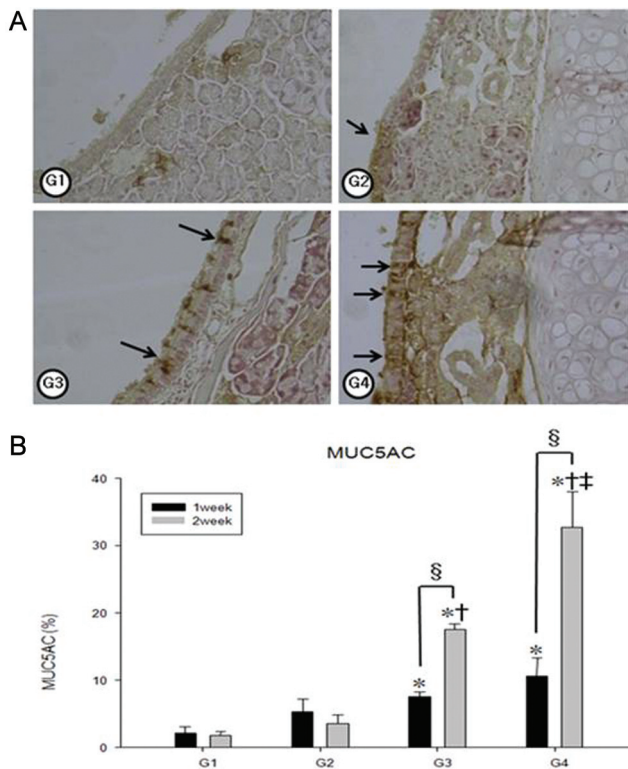


Figure 8. Immunohistochemical staining for MUC5AC in nasal mucosal tissue from mice. The number of MUC5AC-positive cells in the epithelial layer and submucosa was significantly increased in OVA+ASD-treated mice compared with saline-, ASD-, or OVA-treated mice (magnification, 100 $\times$). $\rightarrow$: positive cell. G1: control, G2: ASD, G3: OVA, G4: OVA+ASD. * P < .05 versus the G1 (P = .041, .029 in G3, P = .037, .021 in G4), † P < .05 versus the G2 (P = .038 in G3, P = .023 in G4), ‡ P < .05 versus the G3, § P < .05 1-week period versus 2-week period (P = .033 in G3, P = .020 in G4). % = stained epithelial cells/total epithelial cells $\times$ 100. ASD indicates Asian sand dust; OVA, ovalbumin.

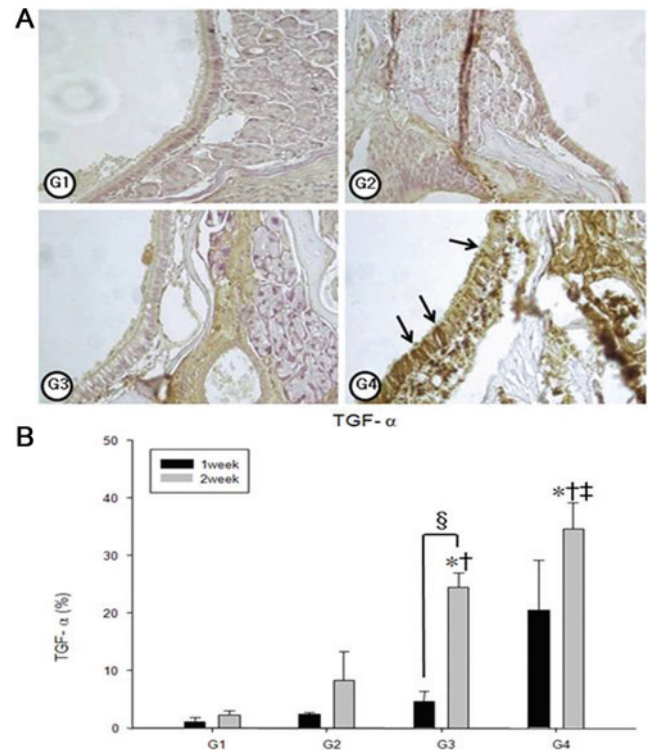


Figure 9. Immunohistochemical staining for transforming growth factor (TGF)- α in nasal mucosal tissue from mice. The number of TGF- α -positive cells in the epithelial layer and submucosa was significantly increased in OVA+ASD-treated mice compared with saline-, ASD-, and OVA-treated mice (magnification, 100 $\times$). $\rightarrow$: positive cell. G1: control, G2: ASD, G3: OVA, G4: OVA+ASD. * P < .05 versus the G1 (P = .035 in G3, P = .029 in G4), † P < .05 versus the G2 (P = .038 in G3, P = .025 in G4), ‡ P < .05 versus the G3, § P = .041 1-week period versus 2-week period. % = stained epithelial cells/total epithelial cells $\times$ 100. ASD indicates Asian sand dust; OVA, ovalbumin.

(24.51% $\pm$ 2.38%; P = .033). However, the number of TGF- α -immunopositive cells was significantly higher at 2 weeks than at 1 week only in the OVA group (P = .041; **Figure 9**).

Discussion

This study demonstrates that *MUC5AC* expression and PAS staining were increased in NCI-H292 cells and in nasal epithelial cells after exposure to ASD. As the concentration of ASD particulate matter increased, the expression of *MUC5AC* mRNA increased in NCI-H292 cells, suggesting that the concentration of particulate matter may be directly related to increased mucus secretion. The decrease in cell viability observed at 500 μ g/mL ASD indicates that cell cytotoxicity developed at this ASD level.

Many stimuli and mechanisms, including bacterial and viral infections, allergic asthma, nasal polyps, cigarette smoke, mechanical stimulation, and inhalation materials, induce mucus hypersecretion.^{15,16} However, the mechanism of ASD-induced mucus secretion is not known. ASD consists of particles that range in size from 0.1 to 20 μ m,

which includes fine particles of less than 2.5 μ m and ultrafine particles of less than 0.1 μ m.^{3,17} Titanium dioxide, a representative ultrafine particle, has been shown to cause many diseases after it is phagocytosed by macrophages. This finding suggests that ASD not only worsens disease states but also causes changes, adaptations, and developments in respiratory cells.¹⁸

Donaldson et al¹⁸ reported that ASD consists of crustal elements and ionic components such as Na⁺, Mg²⁺, Ca²⁺, NH₄⁺, SO₄²⁻, and NO₃⁻. ASD may play a role in activating mucus secretion because it contains toxins such as lipopolysaccharide and heavy metals. However, Park et al¹⁹ proposed that the concentrations of toxins and microbes in ASD are not sufficient to cause disease. Diesel exhaust particulate, 90% of which is ultrafine particles, induces respiratory symptoms such as sputum and cough, and allergic responses are increased in allergic patients with elevated IgE levels after prolonged exposure to diesel exhaust particulate. Although it appears that ASD PM10 may be related to mucus secretion through inflammatory responses in NCI-H292 cells, additional studies are warranted.

The significantly higher number of eosinophils in the nasal mucosa of OVA+ASD mice at 1 and 2 weeks postexposure to ASD in the present study suggests that ASD activated allergic inflammation in the nasal epithelium. Previous studies have proposed that ASD enhances the infiltration of eosinophils and lymphocytes into the airway and increases goblet cell proliferation in the bronchial epithelium.^{8,9,20} ASD and SiO₂ act synergistically to increase the levels of eosinophil-relevant cytokines and chemokines such as interleukin-5 and monocyte chemoattractant protein 3.²¹ This result indicates that allergic inflammation due to ASD may be attributable to its component mineral elements, mainly SiO₂. In the present study, the number of cells positive for MUC5AC and TGF- α was significantly increased in the OVA+ASD mice, implying that ASD-stimulated MUC5AC secretion is associated with increased TGF- α . TGF- α is a ligand of epidermal growth factor receptor, which increases MUC5AC production and induces mucosal secretion through goblet cell proliferation.²² MUC5AC is rarely expressed in healthy epithelium, but it is highly expressed in asthma and polyposis. Based on these results, it is conceivable that MUC5AC is involved in mucus production.²³ In the present study, as the duration of exposure time to ASD increased, the production of MUC5AC and TGF- α also increased. It is presumed that allergic reactions may be increased with prolonged exposure to allergy-triggering factors such as ASD.

The Korean Meteorological Administration has established an Asian Dust Warning System, and advisories are issued when the hourly averaged dust (PM10) concentration is expected to exceed 400 μm^2 for longer than 2 hours. In our experiments, the total amount of sand dust inhaled by mice exposed to 10 mg/mL ASD was very similar to the total amount inhaled by humans in an atmosphere with 400 μm^2 ASD for 24 hours.^{8,20}

The results of the present study show that ASD can induce mucus production in NCI-H292 cells and in nasal epithelial cells. However, this study did not address the mechanism by which PM10 activates inflammatory reactions in these cells or the elements of ASD that cause mucus secretion. Further studies are needed to clarify these issues.

Conclusion

In conclusion, this study shows that ASD, a type of air pollutant in East Asia, can induce mucin production in human respiratory epithelial cells (NCI-H292 cells) and can induce mucin production in murine nasal epithelial cells by activating allergic inflammation. These results suggest that ASD can aggravate airway disease by activating inflammatory reactions through increased mucus secretion.

Author Contributions

Joo Hyun Jung, drafting the article; **Il Gyu Kang**, analysis and interpretation of data; **Heung Eog Cha**, final approval of the

version to be published; **Sung Ho Choe**, acquisition of data; **Seon Tae Kim**, substantial contributions to conception and design.

Disclosures

Competing interests: None.

Sponsorships: None.

Funding source: None.

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