

Increased expression of the aryl hydrocarbon receptor in allergic nasal mucosa, contributing to chemokine secretion in nasal epithelium

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ABSTRACT

Background: Pollutants produced by industrial and traffic-related activities have been linked to allergic responses. These noxious agents induce their effects through the aryl hydrocarbon receptor (AhR).

Objective: We analyzed the expression and distribution pattern of AhR in normal and allergic nasal mucosa, and cytokine-driven regulation of its expression. The production levels of chemokine in cultured nasal epithelial cells were evaluated after stimulation with AhR ligand.

Methods: The expression levels and distribution pattern of AhR in normal, mild, and moderate-severe persistent allergic nasal mucosa were assessed by using real-time polymerase chain reaction, Western blot, and immunohistochemistry. The expression levels of AhR were determined in cultured nasal epithelial cells treated with T-helper 2 cytokines. In cultured epithelial cells stimulated with 2-(10H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester, the expression levels of granulocyte macrophage colony-stimulating factor, thymus and activation regulated chemokine, macrophage inflammatory protein 1 α , monocyte chemoattractant protein 1, regulated on activation normal T-cell expressed and secreted, eotaxin, and interleukin 8 were measured with real-time polymerase chain reaction and enzyme-linked immunosorbent assay.

Results: Expression of AhR was observed in normal and allergic nasal mucosa where it is distributed in the epithelial layer, submucosal glands, endothelial cells, and inflammatory cells. Its expression levels are increased in allergic nasal mucosa and upregulated after stimulation with T-helper 2 cytokines. The stimulation with 2-(10H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester resulted in increased production of chemokines in cultured epithelial cells.

Conclusion: Analysis of the study results indicated that increased expression levels of AhR may play a role in the pathogenesis of allergic rhinitis, which contributes to chemokine production in nasal mucosa.

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Nasal mucosal surfaces are under the constant threat of pathogenic attack and provide an effective barrier against the penetration of pathogens and other environmental harm by protecting the integrity of nasal mucosa, especially nasal epithelial cells, a primary pollutant target that interfaces innate and adaptive immune responses, have been proposed to regulate many of the responses to airborne pollutants.^{1–5}

Allergic rhinitis is a chronic inflammatory condition of the nasal airway and is induced by an immunoglobulin E (IgE) associated immune response to ubiquitous environmental allergens. Its main clinical symptoms are itching, sneezing, rhinorrhea, and nasal obstruction.^{6–8} Although these symptoms are mediated by an IgE-associated pathway, nasal hyperreactivity symptoms are also elicited by nonallergic stimuli, such as change of temperature, smoke, and air pollutants.^{9,10}

A considerable amount of evidence indicates that environmental pollutants can contribute to the occurrence and exacerbation of allergic rhinitis in most industrialized nations.^{11,12} Pollutants, especially particulate matter, that are produced by industrial and traffic-related activities, have been linked to allergic responses.^{13–21} The major components of particulate matter are diesel exhaust, which consists of polycyclic aromatic hydrocarbons and their derivatives with a broad

surface area that can absorb chemicals.^{22,23} These plentiful noxious agents exert their effects through the aryl hydrocarbon receptor (AhR), recognized as a ligand-activated transcription factor.²²

In this respect, we hypothesized that AhR may play an important role in allergic rhinitis. Therefore, the present study was undertaken to (1) investigate the expression levels and distribution pattern of AhR in normal, mild, and moderate-severe persistent allergic rhinitis to evaluate its possible contribution to the pathogenesis of allergic rhinitis, (2) evaluate whether the expression levels of AhR are altered by stimulation of T-helper (Th) 2 cytokines (interleukin [IL] 4, IL-5, and IL-13) relevant to allergic rhinitis by using an epithelial culture system, and (3) elucidate the effect of AhR on the expression of chemokines relevant to allergic rhinitis.

METHODS

This study was carried out at the Korea University College of Medicine. Informed consent was given from all the participants before commencement of the study, and the study protocol was approved by the institutional review board of Korea University College of Medicine (ED14035). Sixty subjects (30 healthy subjects and 60 patients with allergic rhinitis) participated in the study (Table 1). The diagnosis of allergic rhinitis was based on a history of persistent allergic rhinitis, clinical examination, and skin-prick test and provocation test results. The patients with persistent allergic rhinitis were classified according to disease severity, and the severity of rhinitis was classified according to the allergic rhinitis and its impact on asthma reported as mild or moderate-severe.⁶ The patients with allergy and with exacerbation of upper respiratory infection during the 6 weeks preceding the study were not included in the study. Additional criteria for exclusion were a clinical history of asthma; smoking; or recent use of topical or oral steroids, leukotriene antagonists, and antihistamines. Patients undergoing augmentation rhinoplasty were considered healthy control subjects and were required to have been free of nasal or respiratory tract infection, allergy, asthma, smoking, or ongoing drug treatment.

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Table 1 Clinical characteristics of patients

Characteristics	Group A, Normal Control (n = 30)	Group B, Mild Allergic Rhinitis (n = 30)	Group C, Moderate-Severe Allergic Rhinitis (n = 30)
No. women/men	12/18	14/16	15/15
Age, mean (SD), y	35.9 ± 3.3	38.9 ± 6.5	39.9 ± 6.8
Rhinitis duration, y	NA	13.58 ± 2.63	14.22 ± 2.53
Serum total IgE level, mean (SD), IU/mL	25.73 ± 12.25	324.53 ± 132.45	337.14 ± 110.66

SD = standard deviation; NA = not applicable; IgE = immunoglobulin E.

Nasal mucosa was acquired from the inferior turbinate of healthy control subjects and the patients with allergy. By using a Blakesley forceps, the mucosal biopsy sample was taken largely from ~2 cm posterior to the anterior edge of the inferior turbinate. The nasal mucosal samples were divided into two portions. One portion was stored at -70°C for RNA and protein isolation. The other portion was subjected to immunohistochemistry, in which the samples were fixed in 4% paraformaldehyde in phosphate-buffered saline solution, pH 7.4. These samples were then dehydrated in a graded series of ethanol to xylene and embedded in paraffin wax.

Isolation and Culture of Epithelial Cells

Normal nasal mucosal samples were enzymically digested in 0.5% dispase for 24 hours in Dulbecco's Modified Eagle Medium/F-12, including penicillin and streptomycin. Thereafter, the nasal epithelial cells were harvested and cultured. An air-liquid interface was created in bronchial epithelial growth medium (Lonza Walkersville Inc., Walkersville, MD). On reaching 80–90% confluence, the cultured cells were stimulated with cytokines (30 ng/mL of IL-4, IL-5, and IL-13; PeproTech, Inc., Rocky Hill, NJ) for 24 hours or with 2-(10H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) (50 nM/mL; Tocris Bioscience, Bristol, U. K.) for 6, 12, and 24 hours.

Small-Interfering RNA Transfection Experiments

Cultured epithelial cells were used to assess the effects of AhR on the secretion of chemokine in nasal mucosa. The cells were plated in a 6-well culture dish at a density of 5×10^6 cells per well. They were transfected with small-interfering RNA (siRNA) and silencer negative control siRNA (ST Pharm Oligo Center, Si Hwa, Kyung Ki Do, Korea) by using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The efficiency of gene knock down was determined by using reverse transcription polymerase chain reaction (PCR). After transfection, the cells were treated as specified in the Results section.

Total RNA Isolation and Real-time PCR

Total RNAs (1 µg) obtained from nasal mucosal samples and cultured epithelial cells were reverse transcribed in a reaction mixture that contained Molony Murine Leukemia Virus reverse transcriptase (GIBCO BRL, Grand Island, NY). The primer sequences are listed in Table 2. Real-time PCR was performed with an iCycler (BioRad Laboratories, Hercules, CA). Each PCR mixture contained complementary DNA, iTAQ SYBR supermix (Bio-Rad, Hercules, CA), and primers, and the amplification parameters were as follows: 40 cycles of 95°C for 15 seconds, 60°C for 30 seconds, and 72°C for 30 seconds. The level of gene expression was calculated as the difference between the Ct value of the target gene and that of GAPDH used as the control. Relative gene expression was determined by the $2^{-\Delta\Delta C_t}$ method.

Immunocytochemical and Western Blot Analysis

Immunocytochemistry was conducted by using a peroxidase-labeled streptavidin-biotin technique. Briefly, paraffin sections were cut and treated with 0.03% H₂O₂ in methanol for 15 minutes to quench

Table 2 Sequences of PCR primers

Primer	Sequence
AhR	S: 5'-CCAACATCACCTACGCCA G-3' AS: 5'-CCAAACGGTCCAACCTCTGTAT-3'
MCP-1	S: 5'-AAGCAGAAAGTGGGTTTCAGG-3 AS: 5'-ATTCTTGGGTTGTGGAGTGAG-3'
MIP1-α	S: 5'-ACAGTGGTCAGTCCCTTCTTG-3' AS: 5'-AGACCTGCATGATCTGAGC-3'
RANTES	S: 5'-TCCGTTCCCTTTTCATCTCTG-3' AS: 5'-TCTCTTTGTGTTTCCCTTTCCC-3'
Eotaxin	S: 5'-CCAACCACCTGCTGCTTAAACCTG-3' AS: 5'-GCT TTGGAGTTGGAGATTTTGG-3'
GM-CSF	S: 5'-TCTGCTTGTATCCCCCTTG-3' AS: 5'-ATGACCATCTGAGTTTCTAGC-3'
TARC	S: 5'-ATGTGTTAGATGACGGTGG-3' AS: 5'-CCCCAACATTGAGCTTCTTG-3'
IL-8	S: 5'-TTAAATCTGGCAACCCTAGTC-3' AS: 5'-GAGGTAAGATGGTGGCTAATAC-3'

PCR = polymerase chain reaction; AhR = aryl hydrocarbon receptor; S = sense; AS = antisense; MCP-1 = monocyte chemotactic protein 1; MIP1-α = macrophage inflammatory protein 1 α; RANTES = regulated on activation normal T-cell expressed and secreted; GM-CSF = granulocyte macrophage colony-stimulating factor; TARC = thymus and activation regulated chemokine; IL = interleukin.

endogenous peroxidase activity. Thereafter, they were incubated with anti-AhR antibodies at the 1:100 dilutions (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). As a negative control, the primary antibodies were replaced with nonimmune goat IgG (Santa Cruz Biotechnology). The color was developed by using 3,3'-diaminobenzidine.

For Western blot analysis, equal amounts of total protein were separated on 12% sodium dodecyl sulfate-polyacrylamide gels and transferred to Immobilon (Millipore, Bedford, MA). The blots were blocked with PBS-Tween 20 that contained 1% skim milk and then incubated with anti-AhR antibody in PBS-Tween 20 overnight at room temperature. Antibody reactions were detected by using the enhanced chemiluminescence detection kit (Amersham Bioscience, Piscataway, NJ), followed by detection of chemiluminescence on x-ray film. As an internal control, β-actin expression was analyzed in parallel blots by using the β-actin antibody (Sigma, St. Louis, MO). The intensity of detected bands was quantified by using Scion Image Beta 4.0.2 (Scion Corporation, Frederick, MD). Relative intensities of each of the protein signals were obtained by dividing their intensities by those of β-actin.

Analysis of Chemokines by using Enzyme-linked Immunosorbent Assay

The expression levels of granulocyte macrophage colony-stimulating factor, thymus and activation regulated chemokine, macrophage inflammatory protein 1 α, monocyte chemotactic protein 1, regulated on activation normal T-cell expressed and secreted, eotaxin, and IL-8

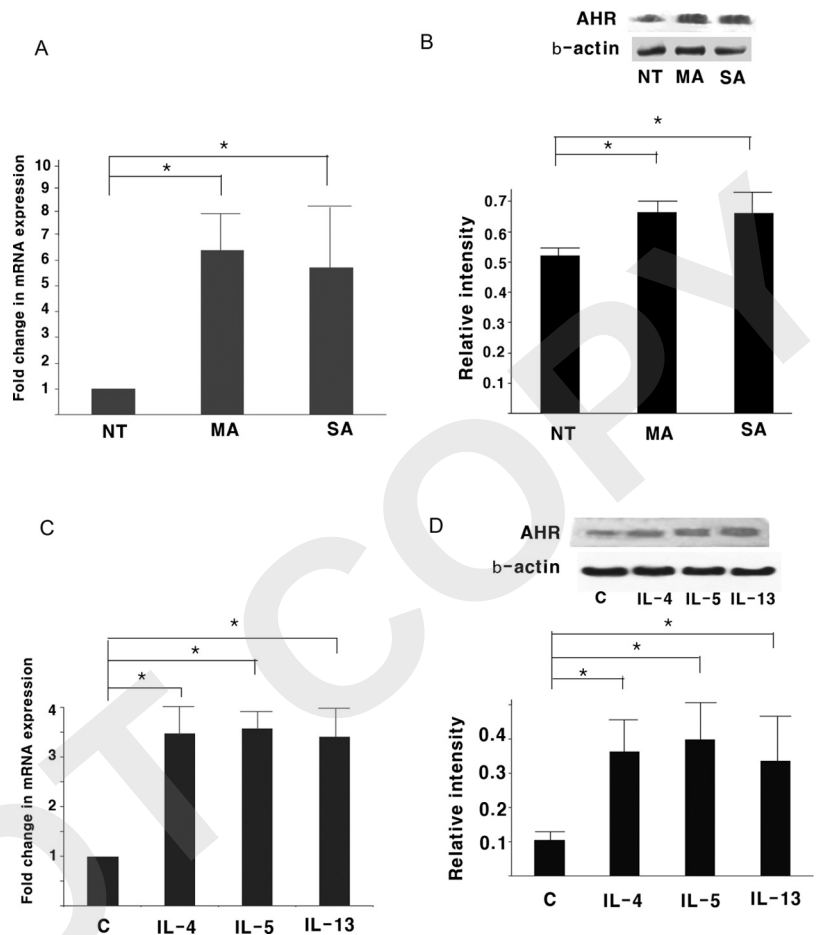


Figure 1. The expression levels of aryl hydrocarbon receptor (AhR) in normal, mild, and moderate-severe persistent allergic nasal mucosa was evaluated by real-time polymerase chain reaction (PCR) (A) and Western blot (B). In cultured cells stimulated with interleukin (IL) 4, IL-5, and IL-13, the expression levels of AhR were analyzed by real-time PCR (C) and Western blot (D). * $p < 0.05$. AHR = Aryl hydrocarbon receptor; MA = mild persistent allergic nasal mucosa; NT = normal nasal mucosa; SA = moderate-severe persistent allergic nasal mucosa.

in supernatants of cultured cells were measured by using enzyme-linked immunosorbent assay by following the manufacturer's protocol (R & D Systems, Abingdon, U.K.). Data were expressed as pg/mL.

Statistical Analyses

Statistical analyses were carried out by using SPSS for Windows (version 16.0.0; SPSS, Chicago, IL). Age differences among the three groups were calculated by one-way analysis of variance, and the Bonferroni *post hoc* correction was applied. The difference in duration of rhinitis between the mild and moderate-severe persistent rhinitis groups was statistically evaluated by using the Mann-Whitney *U* test. A one-way analysis of variance, the Bonferroni *post hoc* correction ($\alpha = 0.0167$), and the Tukey test were performed to evaluate whether values obtained by real-time PCR, Western blot analysis, and enzyme-linked immunosorbent assay differed significantly among each group. The level of significance was set at $p < 0.05$.

RESULTS

The allergy and control groups did not differ by age or male to female ratio. There was no significant difference in the duration of disease between the allergy groups (Table 1). Serum levels of total IgE in allergy groups were higher than in the control group but were not significantly different between the allergy groups (Table 1). Expression of AhR was observed in nasal mucosa of normal subjects and patients with allergic rhinitis, both at messenger RNA (mRNA) and protein levels. However, the expression levels of AhR mRNA transcripts and protein were higher in allergic mucosae than in normal nasal mucosae, but there was no significant difference between both allergy groups (mild and moderate-severe persistent allergic rhinitis)

(Fig. 1). Immunocytochemical results showed that AhR was distributed to epithelial layer, submucosal glands, and endothelial cells as well as inflammatory cells (Fig. 2). However, positive findings were not seen in negative control slides when using nonimmune goat IgG (Fig. 2). The expression levels of AhR after stimulation with Th2 cytokines (IL-4, IL-5, and IL-13) were evaluated by using real-time PCR and Western blots, the results showed increased expression levels of both AhR mRNA and protein compared with nonstimulated cells (Fig. 1, C and D).

After stimulation with ITE, an AhR ligand, the expression levels of macrophage inflammatory protein 1 α , monocyte chemotactic protein 1, regulated on activation normal T-cell expressed and secreted, eotaxin, thymus and activation regulated chemokine, IL-8, and granulocyte macrophage colony-stimulating factor were evaluated by real-time PCR and enzyme-linked immunosorbent assay. Expression levels of each chemokine in cultured cells and supernatants were detectable in nonstimulated control groups. The expression levels generally increased from time 0 over the subsequent time of 6–24 hours after stimulation with ITE (Fig. 3). Furthermore, cells silenced with AhR-specific siRNA did not show any increase in the expression levels of any chemokines tested in the present study after stimulation with ITE (Fig. 3).

DISCUSSION

The AhR has been considered the primary receptor or sensor for various pollutants that are present in automobile exhaust, industrial waste, and cigarette smoke.²⁴ The nasal mucosa is the first tissue that come into contact with pollutants inhaled into the nasal cavity.¹ In this respect, we considered it necessary to perform a detailed analysis of AhR expression in the nasal mucosa of normal controls and pa-

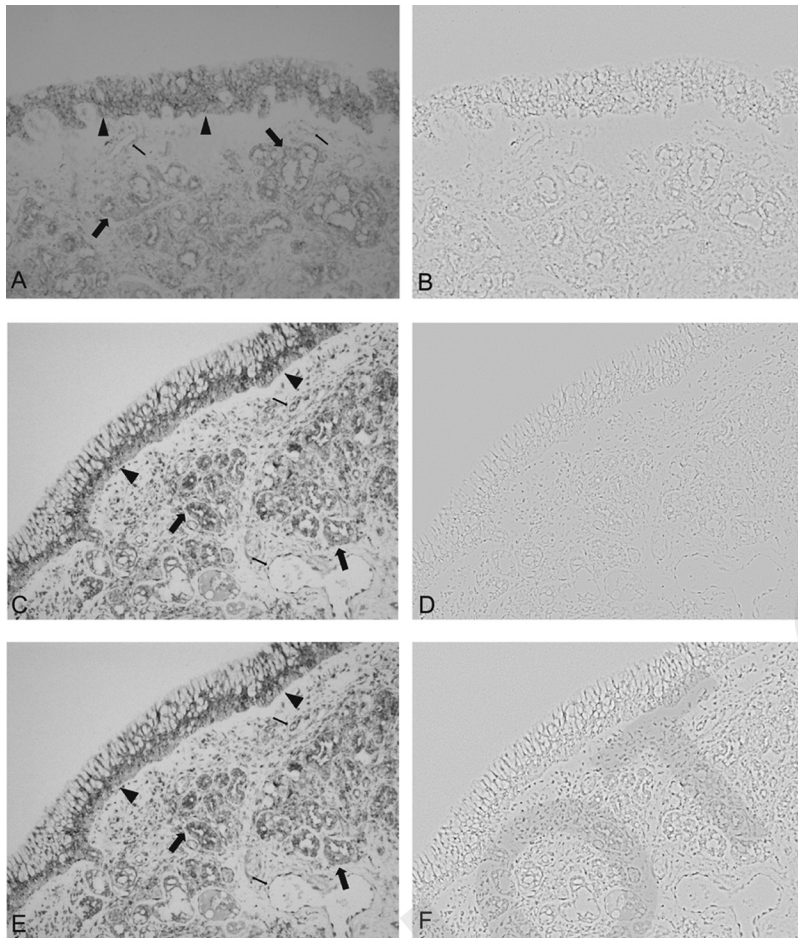


Figure 2. Immunohistochemical localization of aryl hydrocarbon receptor (AhR) in normal (A), mild (C), and moderate-severe persistent allergic nasal mucosa (E). Negative control for AhR in normal (B), mild (D), and moderate-severe persistent allergic nasal mucosa (F). Arrowhead = Superficial epithelial cells; large arrow = submucosal glands; small arrow = endothelial cells.

tients with allergic rhinitis to understand the role of this receptor in allergic rhinitis. Most studies on AhR have been dedicated to its role in mediating the immune response, and AhR has been found to be highly expressed on dendritic cells, Th17 cells, and other immune cells.²⁴ To the best of our knowledge, the expression of AhR on human nasal mucosa has not been addressed before. Interestingly, in this study, we found that AhR is constitutively expressed in normal human nasal mucosa and upregulated in mild and moderate-severe persistent allergic nasal mucosae, where it is distributed in superficial epithelium, submucosal gland, vascular endothelium, and inflammatory cells infiltrated into nasal mucosa. Moreover, its expression levels were upregulated by Th2 cytokines, and its activation by ITE induced the secretion of chemokines in epithelial cells. To study the effect of AhR in epithelial chemokine expression, we knocked down AhR genes of cultured epithelial cells with AhR-specific siRNA and then treated them with ITE. ITE-induced secretion of chemokines was inhibited in cells silenced with AhR-specific siRNA. Taken together, analysis of the results of the current study indicated that AhR may be distributed in resident cells of human nasal mucosa, which participates in the pathogenesis of allergic rhinitis and in controlling the secretion of chemokines in human nasal mucosa.

AhR expression differs significantly among tissues. Its expression levels are constitutively high in liver and in the barrier tissues such as skin, lung, and gut, which are lined by the epithelial layer.²⁵ In these barrier tissues, AhR is highly expressed in epithelial and various immune cells.²⁵ Nevertheless, because AhR is abundant in various cells of the immune system, such as innate lymphoid cells and dendritic cells,^{26–28} the role of AhR in epithelial cells of these barrier tissues has not been sufficiently evaluated. Studies that used AhR agonists demon-

strated that AhR activation induces the production of cytokines such as transforming growth factor α , tumor necrosis factor α , and matrix metalloprotease through its receptors in human pulmonary epithelial cells.^{29–32} Wong *et al.*²⁹ reported that 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) is activated *via* AhR, which induces mucin or matrix metalloprotease production from a Clara-cell-derived cell line and that it is also implicated in the increased expression of cyclooxygenase-2 and IL-1 β mRNA. 1-Nitropyrene and 3-nitrofluoranthene induced a strong increase in several cytokine and chemokine genes, characterized by maximum effects on IL-8 and tumor necrosis factor α expression.^{33–35} These results indicated that activation of AhR localized to the pulmonary epithelial layer may elicit inflammatory action.

To determine the functional role of AhR present in the epithelial layer of human nasal mucosa, we isolated the epithelial cells from human nasal mucosa and cultured them with ITE, an endogenous ligand. Activation of AhR induced increased expression of various chemokines in cultured nasal epithelial cells, such as macrophage inflammatory protein 1 α , monocyte chemoattractant protein 1, regulated on activation normal T-cell expressed and secreted, eotaxin, thymus and activation regulated chemokine, IL-8, and granulocyte macrophage colony-stimulating factor. Therefore, the present results indicated that activation of AhR distributed in the epithelial layer of human nasal mucosa may result in an inflammatory action, which promoted exacerbation of allergic rhinitis by inducing chemokine secretion from epithelial cells. This hypothesis was supported by the results that showed that AhR expression was higher in airway fibroblasts from subjects with asthma and that cockroach-induced transforming growth factor β 1 production in fibroblast was significantly enhanced by one of the major dioxin components, TCDD, which

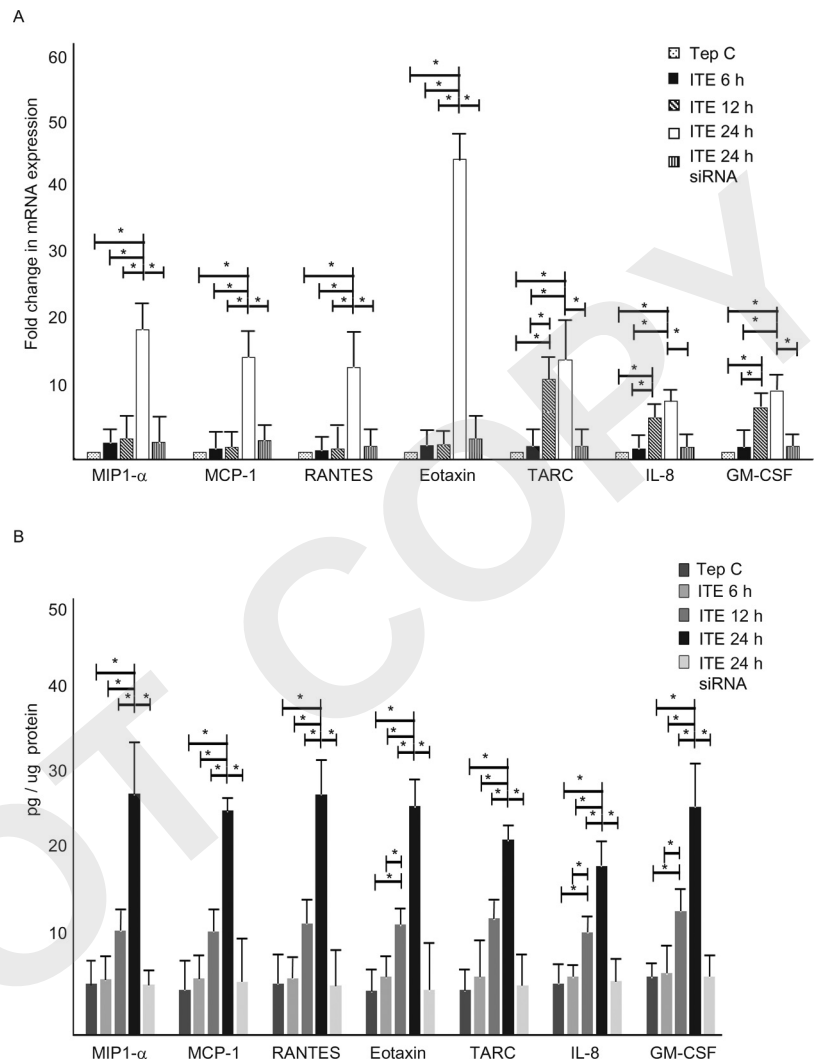


Figure 3. The expression levels of granulocyte macrophage colony-stimulating factor (GM-CSF), thymus and activation regulated chemokine (TARC), macrophage inflammatory protein 1 α (MIP1- α), monocyte chemoattractant protein 1 (MCP-1), regulated on activation normal T-cell expressed and secreted (RANTES), eotaxin, and interleukin (IL) 8 messenger RNA (mRNA), and proteins evaluated by real-time polymerase chain reaction (PCR) (A) and enzyme-linked immunosorbent assay (B). * $p < 0.05$ in statistical significance between 2-(10H-indole-30-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE) stimulated cells and nonstimulated cells. NS = Not significant. ITE 24-hour small-interfering RNA (siRNA) = the expression levels of each chemokine after stimulation with ITE in cells silenced with aryl hydrocarbon receptor (AhR) specific siRNA.

indicated a possible synergistic action between exposure to allergens and environmental chemicals on the development of allergy diseases.^{33,34} Another study also showed that higher AhR levels were found in synovial tissue of rheumatoid arthritis and that TCDD upregulated the expression of IL-1- β , IL-6, and IL-8 through binding to AhR, which indicated its role on the exacerbation of rheumatoid arthritis pathophysiology.³⁵

The AhR has gradually emerged as a regulator of inflammation in various organs and tissues, including lung, skin, and intestine, and its altered levels were associated with various inflammatory diseases.^{36–40} AhR is reported to be involved in the regulation of either pro- or anti-inflammatory responses. For example, in dextran sodium sulfate-evoked colitis, the levels of AhR are increased in the colon epithelium and oral administration of a nontoxic agonist of AhR suppressed the pathogenesis of colitis, which indicated that intestinal epithelial cells expressing AhR are involved in the prevention of colitis.³⁶ Smoke-induced lung inflammation was found to be twofold to threefold more severe in AhR knock out mice than in wild-type mice. In a murine model of colitis, activation of the AhR reduced levels of IL-6, IL-12, interferon γ , and tumor necrosis factor α .³⁷

These results indicated that AhR may have an anti-inflammatory function by suppressing inflammation. However, AhR expression levels in rheumatoid arthritis synovial tissue were higher than those in osteoarthritis synovial tissue.³⁵ In atopic dermatitis and psoriasis, expression of AhR mRNA was increased in lesional skin compared

with that of normal skin from healthy controls and after treatment of normal human epidermal keratinocytes with TCDD, IL-6 and IL-8 production was increased.⁴¹ These results are in accordance with our results that showed that cultured epithelial cells stimulated with ITE show increased expression of chemokines and that AhR expressed in structural cells may be involved in a proinflammatory response. More comparable studies are needed to evaluate the function of AhR in human nasal mucosa. Furthermore, the mechanisms by which AhR expression levels are altered in these tissues are not fully understood.

In the present study, we found that AhR expression levels in nasal mucosa of patients with allergic rhinitis were higher than that in the normal controls. To determine which factors were responsible for this high AhR expression in allergic nasal mucosa, we measured AhR expression levels in cultured epithelial cells treated with Th2 cytokines and found that IL-4, IL-5, and IL-13 upregulated both mRNA and the protein of AhR. These findings indicated that AhR expression in nasal epithelium was stimulated in the presence of Th2 cytokines, which thereby explained the role of Th2 cytokine upregulated in allergic rhinitis. More comparable studies are needed to elucidate the factors that regulate the expression levels of AhR in human nasal mucosa.

CONCLUSION

These results indicated that AhR in human nasal mucosa may function as a primary sensor for pollutants inhaled through the nose,

secrete the chemokines, and participate in the pathogenesis of allergic rhinitis.

REFERENCES

- Seshadri S, Rosati M, Lin DC, et al. Regional differences in the expression of innate host defense molecules in sinonasal mucosa. *J Allergy Clin Immunol* 132:1227–1230.5, 2013.
- Ostrom NK. The history and progression of treatments for allergic rhinitis. *Allergy Asthma Proc* 35(suppl. 1):S3–S10, 2014.
- Kern RC, Conley DB, Walsh W, et al. Perspectives on the etiology of chronic rhinosinusitis: An immune barrier hypothesis. *Am J Rhinol* 22:549–559, 2008.
- Tan BK, Schleimer RP, and Kern RC. Perspectives on the etiology of chronic rhinosinusitis. *Curr Opin Otolaryngol Head Neck Surg* 18: 21–26, 2010.
- Hamilos DL. Chronic rhinosinusitis: Epidemiology and medical management. *J Allergy Clin Immunol* 128:693–707; quiz 708–709, 2011.
- Bousquet J, Van Cauwenberge P, Khaltaev N, et al. Allergic rhinitis and its impact on asthma. *J Allergy Clin Immunol* 108(suppl.):S147–S334, 2001.
- Howarth PH, Salagean M, and Dokic D. Allergic rhinitis: Not purely a histamine-related disease. *Allergy* 55(suppl. 64):7–16, 2000.
- Frieri M. Inflammatory issues in allergic rhinitis and asthma. *Allergy Asthma Proc* 26:163–169, 2005.
- Segboer CL, Holland CT, Reinartz SM, et al. Nasal hyper-reactivity is a common feature in both allergic and nonallergic rhinitis. *Allergy* 68:1427–1434, 2013.
- Gerth van Wijk RG, de Graaf-in't Veld C, and Garred IM. Nasal hyperreactivity. *Rhinology* 37:50–55, 1999.
- Savage J, and Roy D. Allergic rhinitis: An update. *J R Soc Promot Health* 125:172–175, 2005.
- Bauchau V, and Durham SR. Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 24:758–764, 2004.
- Saxon A, and Diaz-Sanchez D. Air pollution and allergy: You are what you breathe. *Nat Immunol* 6:223–226, 2005.
- Jung DY, Leem JH, Kim HC, et al. Effect of traffic-related air pollution on allergic disease: Results of the Children's Health and Environmental Research. *Allergy Asthma Immunol Res* 7:359–366, 2015.
- Huang SK, Zhang Q, Qiu Z, and Chung KF. Mechanistic impact of outdoor air pollution on asthma and allergic diseases. *J Thorac Dis* 7:23–33, 2015.
- Peden DB. Effect of pollutants in rhinitis. *Curr Allergy Asthma Rep* 1:242–246, 2001.
- Peden DB, and Bush RK. Advances in environmental and occupational disorders in 2014. *J Allergy Clin Immunol* 136:866–871, 2015.
- Proietti L, Spicuzza L, and Polosa R. Urban air pollution at the crossroads of the allergic pandemic. *Ann Ital Med Int* 18:64–72, 2003.
- D'Amato G, Liccardi G, and D'Amato M. Environmental risk factors (outdoor air pollution and climatic changes) and increased trend of respiratory allergy. *J Investig Allergol Clin Immunol* 10:123–128, 2000.
- de Marco R, Poli A, Ferrari M, et al. The impact of climate and traffic-related NO₂ on the prevalence of asthma and allergic rhinitis in Italy. *Clin Exp Allergy* 32:1405–1412, 2002.
- Higgins TS, and Reh DD. Environmental pollutants and allergic rhinitis. *Curr Opin Otolaryngol Head Neck Surg* 20:209–214, 2012.
- Okey AB, Riddick DS, and Harper PA. Molecular biology of the aromatic hydrocarbon (dioxin) receptor. *Trends Pharmacol Sci* 15: 226–232, 1994.
- Zhang J, Zhang X, Xia P, et al. Activation of AhR-mediated toxicity pathway by emerging pollutants polychlorinated diphenyl sulfides. *Chemosphere* 144:1754–1762, 2016.
- Esser C, Rannug A, and Stockinger B. The aryl hydrocarbon receptor in immunity. *Trends Immunol* 30:447–454, 2009.
- Esser C, and Rannug A. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacol Rev* 67: 259–279, 2015.
- Wang C, Ye Z, Kijlstra A, et al. Activation of the aryl hydrocarbon receptor affects activation and function of human monocyte-derived dendritic cells. *Clin Exp Immunol* 177:521–530, 2014.
- Wei P, Hu GH, Kang HY, et al. An aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress the Th17 response in allergic rhinitis patients. *Lab Invest* 94:528–35, 2014.
- Song J, Clagett-Dame M, Peterson RE, et al. A ligand for the aryl hydrocarbon receptor isolated from lung. *Proc Natl Acad Sci U S A* 99:14694–14699, 2002.
- Wong PS, Vogel CF, Kokosinski K, and Matsumura F. Arylhydrocarbon receptor activation in NCI-H441 cells and C57BL/6 mice: Possible mechanisms for lung dysfunction. *Am J Respir Cell Mol Biol* 42:210–217, 2010.
- Cheon H, Woo YS, Lee JY, et al. Signaling pathway for 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced TNF- α production in differentiated THP-1 human macrophages. *Exp Mol Med* 39:524–534, 2007.
- Davis JW Jr, Burdick AD, Lauer FT, and Burchiel SW. The aryl hydrocarbon receptor antagonist, 3'-methoxy-4'-nitroflavone, attenuates 2,3,7,8-tetrachlorodibenzo-p-dioxin-dependent regulation of growth factor signaling and apoptosis in the MCF-10A cell line. *Toxicol Appl Pharmacol* 188:42–49, 2003.
- Ishida M, Mikami S, Kikuchi E, et al. Activation of the aryl hydrocarbon receptor pathway enhances cancer cell invasion by upregulating the MMP expression and is associated with poor prognosis in upper urinary tract urothelial cancer. *Carcinogenesis* 31:287–295, 2010.
- Zhou Y, Mirza S, Xu T, et al. Aryl hydrocarbon receptor (AhR) modulates cockroach allergen-induced immune responses through active TGF β 1 release. *Mediators Inflamm* 2014:591479, 2014.
- Frisella PD, Silverberg J, Joks R, and Frieri M. Transforming growth factor beta: A role in the upper airway and rhinosinusitis—*Der-matophagoides pteronyssinus*-induced apoptosis with pulmonary alveolar cells. *Am J Rhinol Allergy* 25:231–235, 2011.
- Kobayashi S, Okamoto H, Iwamoto T, et al. A role for the aryl hydrocarbon receptor and the dioxin TCDD in rheumatoid arthritis. *Rheumatology* 47:1317–1322, 2008.
- Furumatsu K, Nishiumi S, Kawano Y, et al. A role of the aryl hydrocarbon receptor in attenuation of colitis. *Dig Dis Sci* 56:2532–2544, 2011.
- Benson JM, and Shepherd DM. Aryl hydrocarbon receptor activation by TCDD reduces inflammation associated with Crohn's disease. *Toxicol Sci* 120:68–78, 2011.
- Monteleone I, Rizzo A, Sarra M, et al. Aryl hydrocarbon receptor-induced signals up-regulate IL-22 production and inhibit inflammation in the gastrointestinal tract. *Gastroenterology* 141:237–248, 2011.
- Arsenescu R, Arsenescu V, Zhong J, et al. Role of the xenobiotic receptor in inflammatory bowel disease. *Inflamm Bowel Dis* 17:1149–1162, 2011.
- Wang C, Ye Z, Kijlstra A, et al. Decreased expression of the aryl hydrocarbon receptor in ocular Behcet's disease. *Mediators Inflamm* 2014:195094, 2014.
- Kim HO, Kim JH, Chung BY, et al. Increased expression of the aryl hydrocarbon receptor in patients with chronic inflammatory skin diseases. *Exp Dermatol* 23:278–281, 2014. □

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