



Hypoxia disrupts aryl hydrocarbon receptor signaling and the Th17 response in allergic rhinitis patients

Wei Kou, Xuelei Li, Hongbing Yao, Cheng Zhang, Ping Wei*

Department of Otolaryngology, Children's Hospital of Chongqing Medical University, Chongqing, China

ARTICLE INFO

Keywords:

Hypoxia

HIF-1 α

AhR

Allergic rhinitis

ABSTRACT

Background: Hypoxic conditions are a key feature of allergic rhinitis (AR), however, the role of hypoxia in AR remains to be fully understood. The aim of this study was to survey the effect of hypoxia on the Th17 response in AR patients by investigating the action of hypoxia-influenced signaling pathways on Th17 differentiation.

Methods: 23 AR patients and 15 healthy controls were recruited for this study. Under normoxia and hypoxic conditions, the expression of HIF-1 α , AhR, CYP1A1 and CYP1B1 and the presence of Th17 cells in CD4⁺ T cells were measured. Furthermore, the amount of ARNT combined with either HIF-1 α or AhR was determined after the exposure of 2-(1H-Indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE) with normoxia and hypoxia.

Results: HIF-1 α and AhR expression were higher in CD4⁺ T cells from AR patients than in those from healthy controls. In a hypoxic environment, the expression of HIF-1 α was elevated in CD4⁺ T cells of both AR patients and healthy controls. Meanwhile, the suppressive effects of a non-toxic AhR ligand (ITE) on the Th17 response and its positive effects on IL-10 production were suppressed in the cells of AR patients and healthy controls under hypoxia. These effects were arisen from HIF-1 α out-competing AhR for ARNT binding which limited the activity of the AhR pathway.

Conclusions: The present results suggest that hypoxia is capable of promoting the Th17 response by reducing AhR activity via HIF-1 α activity. Thus hypoxia may be intimately involved in the pathogenesis of AR.

1. Introduction

Allergic rhinitis (AR), as an inflammatory airway disease, has a close relationship with hypoxia, which impacts quality of life, sleep, and work performance (Seidman et al., 2015; Cheng et al., 2016). Due to the increasing prevalence of AR, it is rightly called the 21st century epidemic as well (Zvezdin et al., 2015). Thus, it is essential to investigate how hypoxia affects the inflammatory processes associated with AR and to further define the underlying therapeutic targets.

Hypoxia-inducible factor 1 α (HIF-1 α), regarded as the primary environmental sensor of oxygen scarcity, forms heterodimers by binding to aryl hydrocarbon receptor nuclear translocator (ARNT) to drive body inflammation in response to reduced oxygen tension (Pan et al., 2012; Mandl et al., 2016). Since the aryl hydrocarbon receptor (AhR) also binds the same partner (ARNT) to mediate responses to environmental chemicals, it stands to reason that the processes downstream of hypoxia/HIF-1 α and AhR signaling may intersect in AR patients. Our recent studies demonstrate that AhR plays an important role in the

development of AR and that AhR activation by its nontoxic ligand, 2-(1H-Indol-3-ylcarbonyl)-4-thiazolecarboxylic acid methyl ester (ITE) (Wei et al., 2014a,b) can attenuate Th17 responses that are capable of suppressing AR.

In this study, we test the notion that AhR-hypoxia signaling cross-talk may mediate the inflammatory reactions in AR patients. In an effort to better understand ITE-induced AhR target gene expression and the varied robustness of Th17 responses seen at different oxygen levels, we exposed the CD4⁺ T cells from AR patients to ITE in the presence of normal (atmospheric) oxygen levels (20% O₂) or hypoxia (1% O₂). Our results showed that hypoxia significantly interfered with AhR signaling and could disorder expression of AhR target genes through HIF-1 α . Hypoxia also exacerbated the Th17 response and reversed ITE-mediated suppression of Th17 response in CD4⁺ T cells from AR patients and healthy controls.

* Corresponding author at: Department of Otolaryngology, Children's Hospital of Chongqing Medical University, Chongqing, 136[#] Zhongshan 2 Rd., Chongqing, 400014, China.

E-mail address: weiweidoctor@sina.cn (P. Wei).

<https://doi.org/10.1016/j.molimm.2018.07.025>

Received 13 March 2018; Received in revised form 29 June 2018; Accepted 18 July 2018

0161-5890/© 2018 Published by Elsevier Ltd.

2. Materials and methods

2.1. Patients

The Ethics Committee of Chongqing Medical University approved this study (Permit Number: 201522) and all subjects and their guardians provided their written informed consent to participate in the study. 23 AR patients (13 female, 10 male) and 15 healthy controls (8 female, 7 male) were included in the study. The patients were enrolled in the study on the basis of positive skin prick test or serum allergen-specific IgE, and the presence of nasal symptoms according to validated criteria (Pawankar et al., 2012). The skin pricks test and serum allergen-specific IgE was performed according to the guidelines of the European Academy of Allergy and Clinical Immunology (Dreborg et al., 1989). Patients with rhinosinusitis, asthma, or systemic diseases were excluded.

2.2. Cell isolation and culture

We collected 10 mL of heparinized blood from each subject. Peripheral blood mononuclear cells (PBMCs) were isolated from these samples using ficoll-hypaque density-gradient centrifugation. CD4⁺T cells were isolated from PBMCs using human CD4 microbeads (Miltenyi Biotec, CA, USA). CD4⁺T cells were cultured in growth media (RPMI 1640) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, and were maintained in a humidified 37 °C incubator with 20% O₂ (normal oxygen) or with a 1% O₂, 94% N₂ and 5% CO₂ certified gas mixture (hypoxia). To explore the effects of hypoxia on HIF-1 α expression and AhR transcriptional responses in AR patients, CD4⁺T cells were incubated under hypoxia for 24 h on day 3 of culture or for 16 h prior to ITE treatment (100 nM/mL, Tocris Bioscience, USA) and were kept at 1% O₂ for the duration (8 h) of the treatments in the presence of anti-CD3/CD28 (2 μ g/mL; eBioscience, USA) antibodies. Then, the cells were harvested for western blot analysis.

To detect the influence of hypoxia on the Th17 responses of AR patients, CD4⁺T cells were incubated in hypoxic conditions for 16 h prior to ITE treatment (100 nM/mL, Tocris Bioscience, USA) and were kept at 1% O₂ for the duration (8 h) of the treatments in the presence of IL-6 (20 ng/mL; R&D, USA), transforming growth factor (TGF)- β 1 (2 ng/mL; R&D, USA and anti-CD3/CD28 antibodies. The supernatants were harvested for measurement of IL-17 by ELISA, and the cells were harvested for flow cytometry analysis. To study the effect of hypoxia on the production of IL-10 by CD4⁺T cells, these cells were incubated in 1% O₂ for 16 h prior to ITE treatment (100 nM/mL) and were kept under these hypoxic conditions for the 8 h duration of the treatments. T cell activation and ITE treatments were carried out in the presence of transforming growth factor (TGF)- β 1 (2 ng/mL; R&D, USA) and anti-CD3/CD28 antibodies. The supernatants were harvested for an IL-10 ELISA.

2.3. RT-qPCR

RT-qPCR was performed to analyze the mRNA expression of HIF-1 α , AhR, CYP1A1 and CYP1B1. RNA was extracted from CD4⁺T cells using a Total RNA Extraction Kit (Biotek, China) and was reverse transcribed to cDNA using a Prime Script RT Reagent Kit (TaKaRa, China) according to the manufacturer's instructions. RT-qPCR was performed on a 7500 ABI real-time PCR System (Applied Biosystems Inc, Foster City, CA, USA) using a SYBR real-time PCR Premixture Kit (Biotek). Levels of HIF-1 α , AhR, ARNT, CYP1A1, and CYP1B1 mRNA were normalized to the expression of β -actin mRNA. RT-qPCR was performed with the following primers: HIF-1 α forward: 5'-TGC TCA TCA GTT GCC AT T-3', reverse: 5'-TGG GCC ATT TCT GTG TGT A-3'; AhR forward: 5'-TAC TCC ACT TCA GCC ACC ATC-3', reverse: 5'-TAT GGG ACT CGG CAC AAT AAA-3'; ARNT forward 5'-GCT GCT GCT GCC TAC CCT AGT CTC A-3', reverse: 5'-GCT GCT GCT GTC TGG AAT TGT-3', CYP1A1 forward: 5'-

CAC CAT CCC CCA CAG CAC-3', reverse: 5'-ACA AAG ACA CAA CGC CCC TT-3'; CYP1B1 forward: 5'-GCT GCA GTG GCT GCT CCT-3', reverse: 5'-CCC ACG ACC TGA TCC AAT TCT-3'; and β -actin forward: 5'-TGA CGT GGA CAT CCG CAA AG-3', reverse: 5'-CTG GAA GGT GGA CAG CGA GG-3'. The mRNA expression level was determined by finding the $2^{-\Delta\Delta CT}$ values.

2.4. Western blotting

Normalized numbers of CD4⁺T cells were lysed with Radio-Immunoprecipitation Assay (RIPA) lysis buffer. Equal amounts of protein were loaded and separated on sodium dodecyl sulfate-polyacrylamide gels, transferred onto polyvinylidene difluoride membranes and incubated with anti-HIF-1 α antibody (Abcam, UK), anti-AhR antibody (Sigma-Aldrich, USA), anti-ARNT antibody (Sigma-Aldrich, USA), anti-CYP1A1 antibody (Abcam, UK), anti-CYP1B1 antibody (Abcam, UK) or anti- β -actin (Beyotime) primary antibodies. The protein bands were detected with enhanced chemiluminescence. ImageJ software was used to quantify the relative expression levels of the proteins.

2.5. Flow cytometry analysis

To explore the differentiation of Th17, CD4⁺T cells were pre-treated with phorbol myristate acetate (PMA 1 μ g/mL; Alexis Biochemicals, CA, USA) and ionomycin (1 μ g/mL; Sigma-Aldrich, USA) in the presence of monensin (1 μ g/mL; BD, USA) for 8 h (Shen et al., 2011). Activated cells were washed twice and incubated with an FITC-conjugated anti-human CD4 antibody (eBioscience, USA). The cells were then washed, fixed, and permeabilized using a Cytofix/Cytoperm Kit (eBioscience, USA) according to the manufacturer's instructions. The cells were stained with a PE-conjugated anti-human IL-17 A antibody (eBioscience, USA). A PE-conjugated human IgG antibody was used as an isotype control. Data were analyzed using a FACSscan cytometer equipped with FLOWJO software (BD, USA).

2.6. ELISA

IL-17 and IL-10 in the supernatants were detected using a Human DuoSet ELISA Development Kit (R&D, USA) according to the manufacturer's instructions.

2.7. Glutathione-S-Transferase (GST) pull-down assays

Purified GST, GST-ARNT, HIF-1 α and AhR recombinant protein were purchased from Creative BioMart (USA). Different amount of purified HIF-1 α and AhR recombinant protein were incubated with 1 μ g of the affinity purified GST-ARNT protein as indicated in the presence of 0.2% BSA in BC100 (20 mM Tris pH7.9, 100 mM NaCl, 10%glycerol, 0.2 mM EDTA, 0.1% Triton X-100) on a rotator overnight at 4 °C. The proteins were pulled down using GST beads, followed by five times washes with BC100 before elution with 50 μ L of BC100 plus 20 mM reduced glutathione for 10 min. with gentle rotation. Eluted materials were resolved on SDS-PAGE, followed by Western Blotting with the indicated antibodies.

2.8. Statistical analysis

A Student's *t*-test and the nonparametric Mann-Whitney test were applied using SPSS 24 software to determine the significance of observed differences. Data are shown as the mean $\pm$ standard deviation (SD). *P*-values less than 0.05 were considered significant.

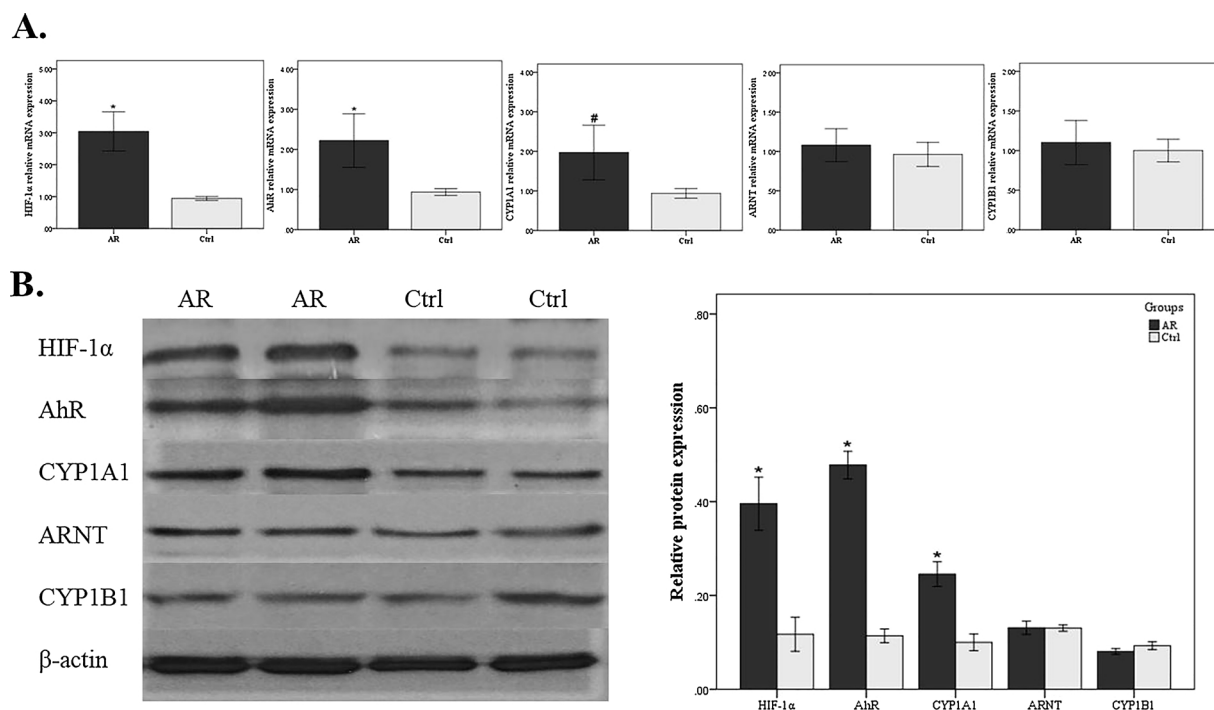


Fig. 1. Expression of HIF-1 α , AhR and CYP1A1 in CD4⁺T cells from AR patients is higher than in those from controls. But no significant difference of ARNT and CYP1B1 expression was observed between AR patients and controls. (A) The mRNA expression of HIF-1 α , AhR and CYP1A1 in AR patients and controls (n = 9) were measured by RT-qPCR. (B) The protein expression of HIF-1 α , AhR and CYP1A1 in AR patients and controls were detected by western blotting (n = 6). Values represent mean $\pm$ standard deviation. *P < 0.01, #P < 0.05.

3. Results

3.1. Increased expression of HIF-1 α , AhR and CYP1A1 in AR patients, but not ARNT and CYP1B1 in AR patients relative to controls

In order to explore the relationship between the hypoxia and aryl hydrocarbon receptor signaling, RT-qPCR and western blotting were used to detect the mRNA and protein expression of HIF-1 α , AhR and CYP1A1. We found that the HIF-1 α , AhR and CYP1A1 mRNA and protein expression were dramatically higher in CD4⁺T cells from AR patients than in those from controls. However, no significant differences in either the mRNA or protein levels of ARNT or CYP1B1 were observed between AR patients and controls (Fig. 1).

3.2. Hypoxia increases HIF-1 α and CYP1A1 expression in both AR patients and controls; but there is no significant change of CYP1A1 expression in AR patient-derived cells which exposure to 1% O₂ prior to ITE treatment compared to these cells treated with ITE only or treated with hypoxia only

To explore the influence of hypoxia on HIF-1 α expression and ITE induced AhR transcriptional responses in AR patients, we examined HIF-1 α , AhR and CYP1A1 mRNA and protein levels in CD4⁺T cells activated under normoxia, hypoxia, those treated with ITE under normoxia, and those were perturbed by hypoxia prior to ITE treatment. The expression of HIF-1 α and CYP1A1 increased after exposure to 1% O₂ in AR patients and controls. ITE effectively upregulated AhR expression in controls, and CYP1A1 expression in both AR patients and controls. There was no difference of HIF-1 α expression in the ITE-treated cells and AhR expression in the hypoxia-treated cells from AR patients and controls. Furthermore, exposure to 1% O₂ prior to ITE treatment didn't alter the degree of CYP1A1 expression by AR patient-derived cells compared to cells treated with ITE under normal oxygen levels only or exposure to hypoxia only (Fig. 2).

3.3. Hypoxia exacerbates the Th17 response and negates the suppression of Th17 response by ITE in AR patients

Our previous study showed that ITE inhibited inflammatory and allergic responses by suppressing elements of the Th17 response *in vitro* (Wei et al., 2014a,b). To evaluate the influence of hypoxia on Th17 response, we assessed the indicator of Th17 cell differentiation (i.e. IL-17 production) in CD4⁺T cells incubated under hypoxia only or hypoxic conditions for 16 h prior to an 8-h ITE treatment. IL-17 expression by CD4⁺T cells was determined by flow cytometry and ELISA. The results revealed that hypoxia aggravated the Th17 response and negated the ability of ITE treatment to suppress IL-17 production by CD4⁺T cells from AR patients and controls (Fig. 3).

3.4. Hypoxia inhibits the IL-10 expression and suppresses the ITE-mediated potentiation of IL-10 production in AR patients

As IL-10 has important anti-inflammatory roles, we examined the levels of IL-10 in the supernatants of CD4⁺T cells from AR patients and controls. Production of IL-10 in CD4⁺T cells from AR patients was markedly lower than in CD4⁺T cells from controls (Fig. 4A). Then, we examined the IL-10 levels in the supernatants of CD4⁺T cells that were treated with ITE after hypoxic culture and those from cells treated with ITE or hypoxia respectively. Hypoxia or hypoxia prior to ITE treatment both reduced the production of this anti-inflammatory cytokine compared to ITE only controls. Similar trends were observed in both AR patients and controls (Fig. 4B).

3.5. HIF-1 α competes with AhR for ARNT binding and limits the activity of the AhR pathway in AR patients

To measure the affinity of ARNT combined with HIF-1 α or AhR, we examined the quantity of HIF-1 α /ARNT and AhR/ARNT heterodimers by GST pull-down assay.

Our findings suggested that HIF-1 α occupies a larger fraction of the

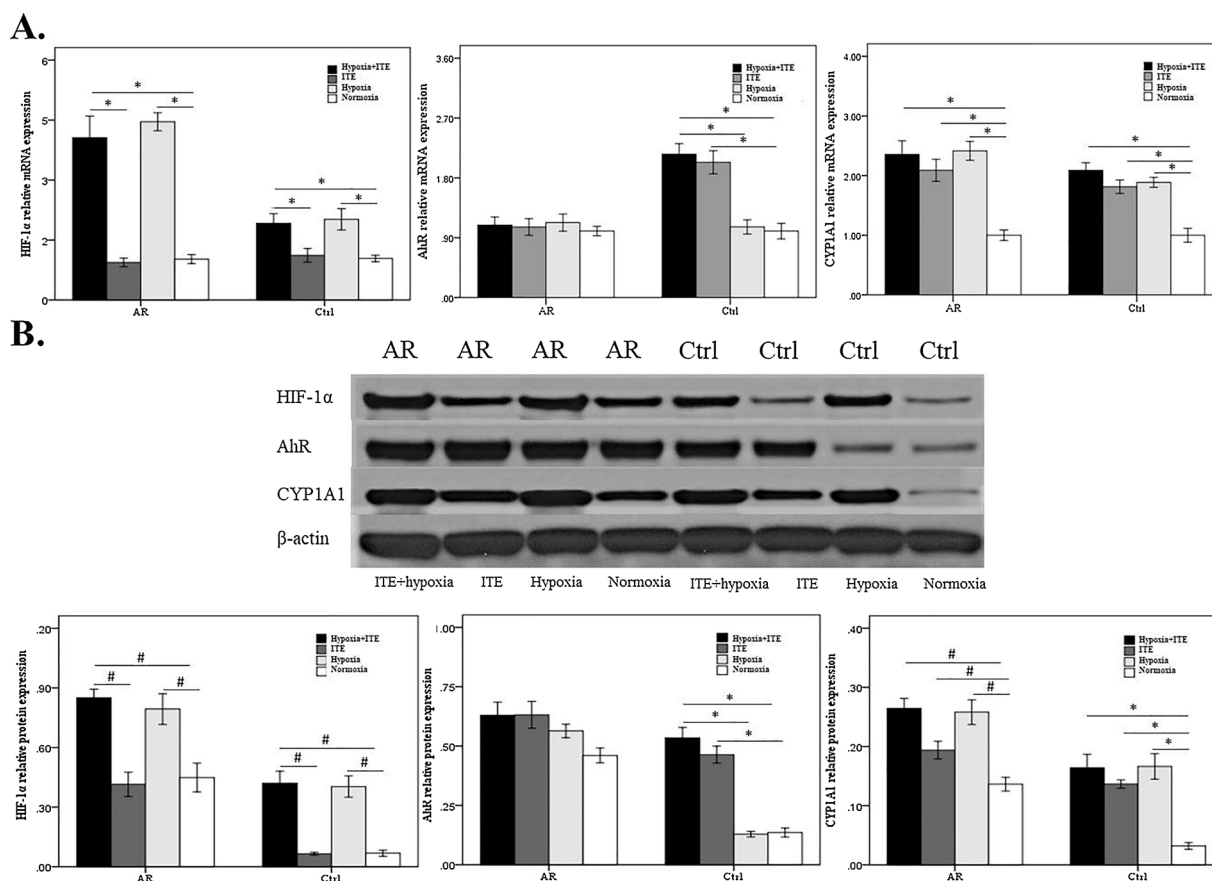


Fig. 2. Hypoxia promotes HIF-1 α and CYP1A1 expression in both AR patients and controls, ITE treatment increases AhR expression in controls, and CYP1A1 in both AR patients and controls. But no significant change of CYP1A1 expression in AR patient-derived cells which exposure to 1% O₂ prior to ITE treatment compared to these cells treated with ITE only or hypoxia only. (A) RT-qPCR analysis of HIF-1 α , AhR and CYP1A1 mRNA expression in CD4⁺T cells after treatment (n = 4). (B) Western blotting analysis of HIF-1 α , AhR and CYP1A1 protein expression in CD4⁺T cells after interference (n = 4). Values represent mean $\pm$ standard deviation.*P < 0.01, #P < 0.05.

ARNT protein pool at the same concentration with AhR; HIF-1 α has a remarkably higher affinity for ARNT binding (Fig. 5).

4. Discussion

Hypoxia and inflammation are intimately linked. (Wanderer, 2011; Cho and Kim, 2018). A growing body of research has demonstrated that hypoxia is a well-characterized trigger of HIF-1 α -dependent signaling to contribute to allergic immune responses and bronchial asthma (Dewitz et al., 2017; Byrne et al., 2013). In our present study, we found that hypoxia alters AhR signaling and Th17 responses in AR patients through the HIF-1 α -ARNT pathway. Firstly, we showed that both HIF-1 α and AhR levels are up-regulated in AR patients. Secondly, under hypoxic conditions, HIF-1 α competes for, and binds with ARNT to the apparent detriment of AhR activity and the transcriptional response under its control. Lastly, while hypoxic conditions associated with inflammation can promote Th17 differentiation and IL-17 production, it also suppressed expression of the anti-inflammatory cytokine IL-10 in this study. These results strongly suggest that hypoxia is capable of reducing AhR activity, and they implicate HIF-1 α as a regulatory factor intimately involved in the pathogenesis of AR.

HIF-1 α activity can be triggered by microorganisms, cancer, and allergies in addition to hypoxic conditions (Olson and van der Vliet, 2011; Huerta-Yepez et al., 2011). This factor usually dimerizes ARNT to regulate immune cell metabolism and direct immune functions. These include driving T cell differentiation and promoting pro-inflammatory factors expression, both of which play important roles in immune defense. Moreover, recent researches suggest that HIF-1 α activation is

critical for maintaining human allergic effector cell survival and function. Zhou. et al. found that HIF-1 α levels were raised in the nasal mucosa of mice with AR and could be attenuated by the HIF-1 α inhibitor 2ME2 (Zhou et al., 2012). Importantly, Mo. et al. also revealed increased HIF-1 α expression in the nasal mucosa of AR patients, suggesting that this molecule plays an important role in the disease. However, little is known about the exact role of HIF-1 α in AR (Mo et al., 2014).

AhR is a cellular receptor that recognizes a variety of ligands including ITE. After activation by its ligands, AhR translocate to the nucleus and binds with ARNT to exhibit strong immunomodulation potential via regulation of the differentiation of T-cells and DCs (Esser and Rannug, 2015). Because HIF-1 α and AhR share the same dimerization partner, it is possible that, if activated simultaneously, they may compete for the shared pool of ARNT and thereby mutually interfere with each other's actions.

In this study, levels of both HIF-1 α and AhR in the CD4⁺T cells of AR patients were substantially elevated. In addition, AhR-mediated CYP1A1 enzyme activity was also increased in AR disorders compared to controls, showing that HIF-1 α and AhR may be activated in AR patients. To further ascertain whether hypoxia-triggered signaling plays a critical role in AR, we measured the activity of HIF-1 α and AhR. The measured abundance of these factors suggested that HIF-1 α levels were significantly increased by hypoxia, but the activation of AhR in response to ITE treatment was significantly reduced under hypoxic conditions. Moreover, these conditions may cause HIF-1 α to sequester ARNT and thus inhibiting the activation of a robust AhR transcriptional response in response to an AhR ligand. Thus, exposure to AhR ligands

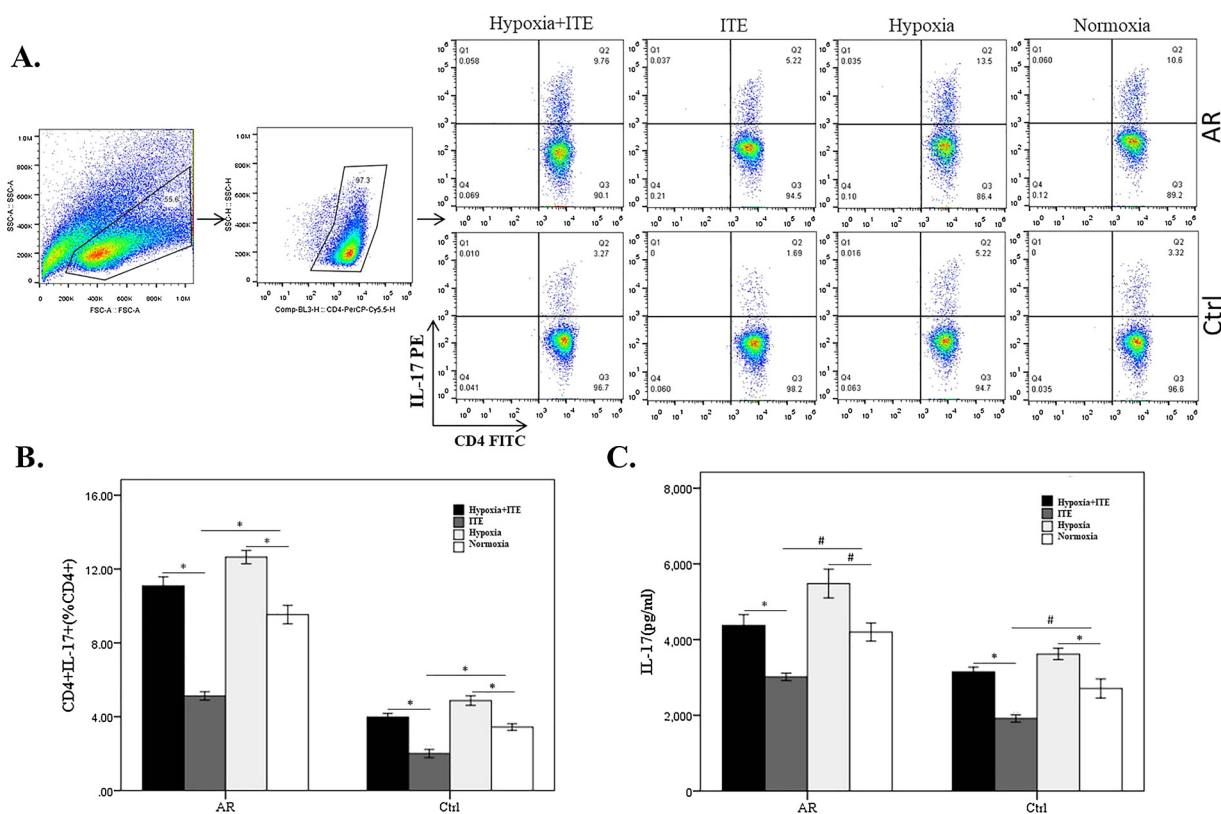


Fig. 3. Hypoxia aggravates Th17 response and inhibits the ITE suppressive effect on Th17 response in CD4⁺ T cells from AR patients and controls (n = 3). (A) Flow cytometry analysis of intracellular IL-17 expression in CD4⁺ T cells. (B) Quantitation analysis of the percentage of Th17⁺ cells. (C) Detection of IL-17 levels in the supernatants by ELISA. Values represent mean $\pm$ standard deviation. *P < 0.01, #P < 0.05.

may not be able to attenuate HIF-1 α -mediated responses when oxygen is scarce.

Indeed, in agreement with previous reports, we found an association between hypoxia and Th17 differentiation and enhanced IL-17 production. Our observations in cells of AR patients indicate that HIF-1 α activity could represent a main mechanism by which hypoxic conditions associated with inflammation can perpetuate the generation, function of Th17 cells in this disease. In our previous research, we found that ITE acts on CD4⁺ T cells to inhibit the Th17 response-a process that could suppress AR through AhR (Wei et al., 2014b).

Conversely, hypoxia appears to reverse ITE-mediated suppression of Th17 immunity among the CD4⁺ T cells from AR patients, demonstrating that hypoxia may be capable of reducing AhR activity and directly influencing the anti-inflammatory effects of ITE. Besides, it has been reported that under hypoxia in humans, regulatory T (Treg) cell functions may be reduced as evidenced by suppression of IL-10 production (Dang et al., 2011). Our studies showed that hypoxia restrains the IL-10 production from CD4⁺ T cells, which may mean hypoxia is a major player in modulating the Th17/Treg balance in AR, and inhibiting inflammatory hypoxia is vital in treating AR patients.

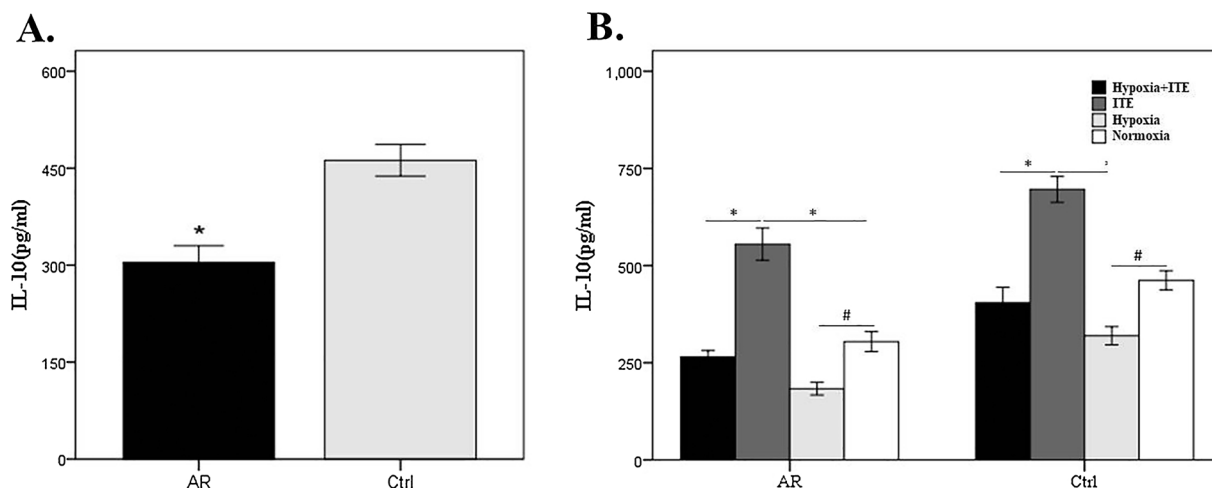


Fig. 4. Hypoxia inhibits IL-10 expression and suppressed the ITE positive effect on IL-10 production in AR patients (n = 3). (A) Production of IL-10 in CD4⁺ T cells from AR patients was higher than in CD4⁺ T cells from controls. (B) Treatment with hypoxia only or hypoxia prior to ITE reduced IL-10 release compare to only treat by ITE in AR patients and controls. ELISA analysis of IL-10 production in the supernatants of CD4⁺ T cells from AR patients and controls. Values represent mean $\pm$ standard deviation. *P < 0.01, #P < 0.05.

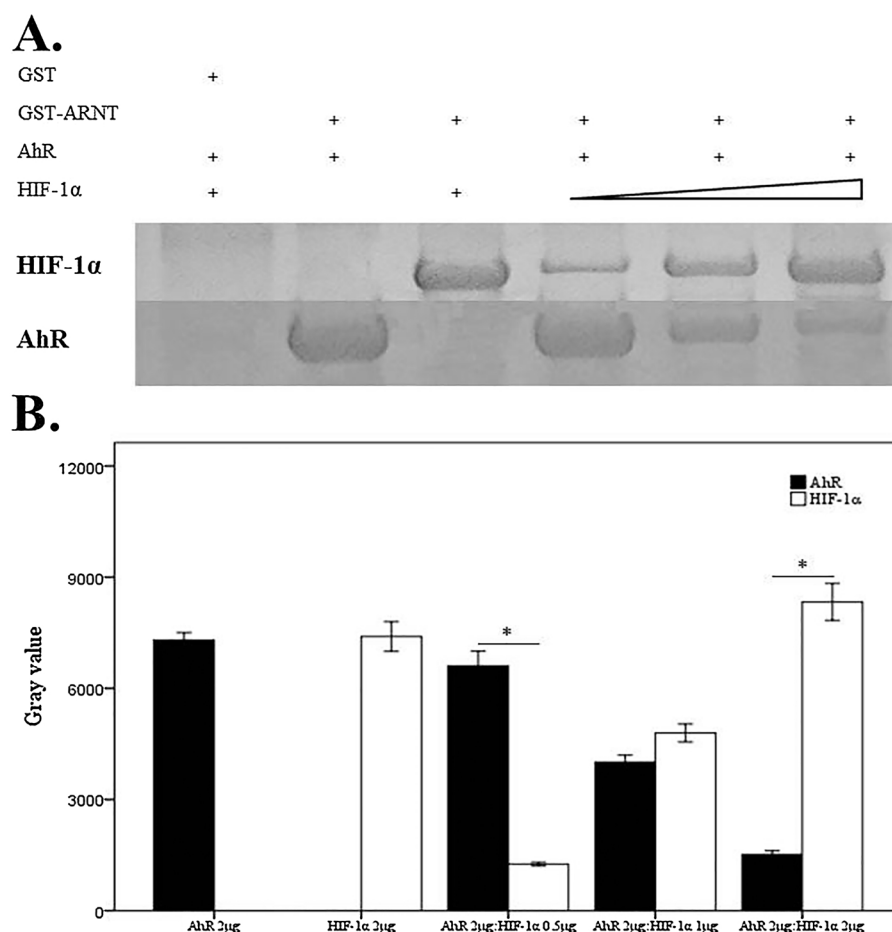


Fig. 5. The binding affinity between HIF-1 α and ARNT was higher than that between AhR and ARNT ($n = 3$). (A) Different amount (0, 0.5, 1 and 2 μ g) of purified HIF-1 α and 2 μ g AhR recombinant protein were analyzed by GST pull-down assay and Western Blotting. (B) Quantitation analysis of the gray value of HIF-1 α and AhR. Values represent mean $\pm$ standard deviation. * $P < 0.01$, # $P < 0.05$.

The HIF-1 α and AhR proteins are critical factors that sense and respond to low oxygen environments, respectively. Through their common cofactor ARNT a regulatory crosstalk exists that can affect AhR and HIF-1 α -mediated responses. There is an antagonistic relationship between AhR and HIF-1 α signaling under conditions of oxygen deprivation that stabilize HIF-1 α levels. This interplay can affect cellular responses mediated by the AhR in AR patients. Hypoxia and inflammation are intertwined at the molecular and cellular levels. Oxygen-sensing mechanisms and hypoxia signaling are potential therapeutic targets for the treatment of AR illness.

Disclosure

The authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

Funding

The study supported by National Natural Science Foundation Project Grant81600781, Chongqing Medical University Science Foundation for Fostering Talents Grant201405 and Project of Science and Technology Plan of Chongqing Yuzhong District Grant20150118.

References

- Byrne, A.J., Jones, C.P., Gowers, K., et al., 2013. Lung macrophages contribute to house dust mite driven airway remodeling via HIF-1 α . *PLoS One* 8 (7), e69246.
- Cheng, K.J., Bao, Y.Y., Zhou, S.H., 2016. The role of hypoxia inducible factor in nasal inflammations. *Eur. Rev. Med. Pharmacol. Sci.* 20 (24), 5067–5076.
- Cho, H.J., Kim, C.H., 2018. Oxygen matters: hypoxia as a pathogenic mechanism in Rhinosinusitis. *BMB Rep.* 25 pii: 4085.
- Dang, E.V., Barbi, J., Yang, H.Y., et al., 2011. Control of T(H)17/T(reg) balance by

- hypoxia-inducible factor 1. *Cell* 146 (5), 772–784.
- Dewitz, C., McEachern, E., Shin, S., et al., 2017. Hypoxia-inducible factor-1 α inhibition modulates airway hyperresponsiveness and nitric oxide levels in a BALB/c mouse model of asthma. *Clin. Immunol.* 176, 94–99.
- Dreborg, S., Backman, A., Basomba, A., 1989. Skin tests used in type I allergy testing position paper. *Allergy* 44 (Suppl. 10), 1–59.
- Esser, C., Rannug, A., 2015. The aryl hydrocarbon receptor in barrier organ physiology, immunology, and toxicology. *Pharmacol. Rev.* 67 (2), 259–279.
- Huerta-Yepez, S., Baay-Guzman, G.J., Bebenek, I.G., et al., 2011. Hypoxia inducible factor promotes murine allergic airway inflammation and is increased in asthma and rhinitis. *Allergy* 66 (7), 909–918.
- Mandl, M., Lieberum, M.K., Depping, R., 2016. A HIF-1 α -driven feed-forward loop augments HIF signalling in Hep3B cells by upregulation of ARNT. *Cell Death Dis.* 7 (6), e2284.
- Mo, J.H., Kim, J.H., Lim, D.J., et al., 2014. The role of hypoxia-inducible factor 1 α in allergic rhinitis. *Am. J. Rhinol. Allergy* 28 (2), e100–6.
- Olson, N., van der Vliet, A., 2011. Interactions between nitric oxide and hypoxia-inducible factor signaling pathways in inflammatory disease. *Nitric Oxide* 25 (2), 125–137.
- Pan, F., Barbi, J., Pardoll, D.M., 2012. Hypoxia-inducible factor 1: a link between metabolism and T cell differentiation and a potential therapeutic target. *Oncoimmunology* 1 (4), 510–515.
- Pawankar, R., Bunnag, C., Khaltayev, N., et al., 2012. Allergic rhinitis and its impact on asthma in Asia Pacific and the ARIA update 2008. *World Allergy Organ. J.* 5, S212–7.
- Seidman, M.D., Gurgel, R.K., Lin, S.Y., et al., 2015. Clinical practice guideline: allergic rhinitis. *Otolaryngol. Head Neck Surg.* 152 (1 Suppl), S1–43.
- Shen, Y., Hu, G.H., Yang, Y.C., et al., 2011. Allergen induced Th17 response in the peripheral blood mononuclear cells (PBMCs) of patients with nasal polyposis. *Int. Immunopharmacol.* 12 (1), 235–240.
- Wanderer, A.A., 2011. Hypoxia and inflammation. *N. Engl. J. Med.* 364 (20), 1976.
- Wei, P., Hu, G.H., Kang, H.Y., et al., 2014a. Increased aryl hydrocarbon receptor expression in patients with allergic rhinitis. *QJM* 107 (2), 107–113.
- Wei, P., Hu, G.H., Kang, H.Y., et al., 2014b. An aryl hydrocarbon receptor ligand acts on dendritic cells and T cells to suppress the Th17 response in allergic rhinitis patients. *Lab. Invest.* 94 (5), 528–535.
- Zhou, H., Chen, X., Zhang, W.M., et al., 2012. HIF-1 α inhibition reduces nasal inflammation in a murine allergic rhinitis model. *PLoS ONE* 7 (11), e48618.
- Zvezdin, B., Hromis, S., Kolarov, V., et al., 2015. LinkOut – more resources allergic asthma and rhinitis comorbidity. *Vojnosanit. Pregl.* 72 (11), 1024–1031.