Increased expression of aryl hydrocarbon receptor and interleukin 22 in patients with allergic asthma

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Summary

Objectives: **We sought to determine whether the aryl hydrocarbon receptor (AhR) and interleukin (IL)-22 may be involved in the pathogenesis of the peripheral blood mononuclear cells (PBMCs) in allergic asthmatic patients and whether their expression may be related to the severity of the disease.**

Methods: **Blood samples were obtained from each subject with allergic asthma (n =18), controlled asthma (n =17) and healthy controls (n =12) respectively. The PBMCs were collected for AhR mRNA detection by real-time quantitative polymerase chain reaction (PCR). The plasma was collected for IL-22 protein detection by enzyme-linked immunosorbent assay (ELISA).**

Results: **The expression of AhR mRNA in PBMCs and IL-22 protein in plasma of patients with allergic asthma were higher than those in controlled asthma cases and healthy controls. The plasma concentrations of IL-22 had negative correlation with the predicted percentage of forced expiratory volume in the first second (FEV_1%) and the percentage of FEV_1 and forced vital capacity (FEV_1/FVC%) and it was positively correlated with the asthma severity score (ASS) of the asthmatics.**

Conclusion: **Our results suggested that both AhR and IL-22 might be involved in the pathogenesis of allergic asthma in human and the level of IL-22 might have some relationship with the severity of the disease.** ***(Asian Pac J Allergy Immunol 2011;29:266-72)***

Key words: *aryl hydrocarbon receptor, interleukin 22, interleukin 17, T helper 17 cell, allergic asthma*

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Introduction

Allergic asthma is a chronic inflammatory airway disease, in which many cells (including mast cells, eosinophils, T lymphocytes, macrophages, neutrophils and epithelial cells) and cellular elements are involved in the inflammation. Its pathogenesis is not fully understood. Recent studies have showed that T_H17 cells and their cytokines may play an important role in the pathogenesis of allergic asthma in mouse models and humans[1,2]. In asthmatic patients, the levels of IL-17 mRNA or protein were reported to be increased in the lungs, sputum, bronchoalveolar lavage fluids or sera and the levels of IL-17 were correlated with the degree of severity of airway hypersensitivity in asthmatic patients[3-8]. IL-17 was mainly produced by T_H17 cells. Other studies have also reported that peripheral blood T_H17 cells were markedly increased in patients with allergic asthma[1,9]. This evidence suggests that T_H17 cells and IL-17 may contribute to the pathogenesis of allergic asthma.

IL-22 is also predominantly secreted by T_H17 cells. IL-22 is highly expressed in various infectious or inflammatory diseases such as Klebsiella pneumoniae, hepatitis C virus, psoriasis, rheumatoid arthritis and Crohn's disease[10]. In asthmatic mice models, IL-22 was reported to inhibit the functions of dendritic cells and attenuate the allergic response[11]. In addition, another study found that the concentration of IL-22 in the plasma of patients with allergic asthma was significantly higher than that in healthy subjects[2]. However, whether IL-22 levels are related to a variety of clinical indices which are related to the severity of asthma in patients is still unclear.

AhR is a ligand-activated transcription factor in the cytosol, which is activated by various xenobiotic ligands, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and 6-formylindolo[3,2-b] carbazole (FICZ). Upon combining with the ligands, AhR undergoes a conformational change and exposes a nuclear translocation site and dimerizes with AhR nuclear translocator (Arnt). The AhR /Arnt heterodimer

binds to the xenobiotic responsive element (XRE) in the nucleus, which causes a variety of toxicological effects[12]. Recent studies have found that AhR was highly expressed in human T_H17 cells and that it played some role in inflammation. In the mice model of experimental autoimmune encephalomyelitis (EAE), AhR activation caused deterioration of the disease by promoting T_H17 functional differentiation[13]. This evidence from AhR-deficient mice suggested that AhR may play important physiological roles in the immune system[14-16]. Another study found that T_H17 cells did not produce IL-22 in the AhR-deficient mice, whether FICZ was presented or not[13].

In this study, we investigated whether the level of AhR and IL-22 changed or not in patients with allergic asthma and whether they might be related to the clinical indices of patients with allergic asthma.

Subjects and methods

Subjects

Thirty five subjects were enrolled from outpatients and inpatients of the Department of Respiratory Diseases, Tongji Hospital (Wuhan, China). Subjects were divided into three groups, including allergic asthma (n =18), controlled asthma (n =17) and age-matched healthy controls who were volunteers in our hospital (n =12). We defined allergic asthma and controlled asthma according to the asthma guidelines proposed by the American Thoracic Society criteria[17]. The diagnosis of allergic asthma was established by symptoms of asthma and improvement in the FEV_1 of ≥12% after administration of salbutamol (200ug) or deterioration in the FEV_1 of >20% after using acetylcholine. We considered patients who had reached levels of asthma control after regular treatment as controlled asthma[18]. The patients with allergic asthma had positive skin reactions to at least one of the following allergens: house dust mite, cat fur, dog hair and weed pollen by skin prick tests. Healthy volunteer were subjects without a history of allergy and asthma symptom scores (ASS=0). All subjects had no history of smoking or atopic dermatitis. Subjects who had histories of respiratory tract infection within 6 weeks before the study or who had received immunotherapy in the previous 5 years were excluded. Asthmatic patients were only treated with inhaled β2-agonists and had no oral steroid intake or leukotriene receptor antagonist therapy during the 4 weeks preceding the study. Asthma severity was assessed by ASS using a standard questionnaire[19]. The FEV_1 and FVC of subjects were measured by means of spirometer (Jaeger, Germany）.

The study was approved by Ethics Committee of Tongji Hospital and all subjects were required to sign informed consent.

Methods

Isolation of PBMCs and plasma

Five milliliter samples of peripheral blood were obtained from each of the subjects in the three groups. The PBMCs were isolated from four milliliters peripheral blood using Ficoll-Hypaque density gradient centrifugation. One milliliter plasma samples were collected by centrifugation and preserved at -20°C for later enzyme-linked immunosorbent assay (ELISA) detection.

Real-time PCR

Total RNA was extracted from PBMCs with TRIzol Reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Complementary DNA was synthesized from the total RNA (0.5 μg) using the PrimeScript™ RT regent Kit (TaKaRa Biotechnology, Dalian, China) following the instructions provided by the manufacturer. PCR amplification reactions were conducted with a SYBR Green Supermix (TaKaRa) in a 20 μl reaction volume containing 0.4μl primers, 2.0μl cDNA and ROX Reference Dye 0.4μl. Reactions were carried out on a 7900 real-time PCR System (Applied Biosystems Inc., Foster, USA) for 40 cycles (95°C for 5 s, 60 °C for 30s), after initial 30 seconds incubation at 95°C. All measurements were performed in duplicate. The level of AhR mRNA expression was assessed relative to housekeeping geneβ-actin expression. Real-time PCR were performed with the following primers: AhR forward:5 ' - AATCCAGTACTGCCAGGCC AAC -3 ' , reverse: 5 ' - GGTCTGGCTTCTGACG GATGA -3 ' . β-actin forward: 5 ' -GCAAGCAGG AGTATGACGAG-3 ' , reverse: 5 ' -CAAATAAA GCCATGCCAATC -3 ' . The data were analyzed with the comparative threshold cycle (Ct) method for gene expression relative to housekeeping gene β-actin.

Detection of IL-22 by ELISA

The concentration of IL-22 protein in plasma was measured with an ELISA Kit following the manufacturer's instructions (Bender MedSystems, Vienna, Austria). The detection limit for the IL-22 ELISA was 5 pg/ml. Intra- and inter-assay coefficients of variation for all ELISA were < 6.7%

Table1. Characteristics of all subjects

	Allergic asthma (n=18)	Controlled asthma (n=17)	Healthy controls (n=12)
Age (years)*	42.22±13.73	36.76±12.43	39.50±10.92
Sex(male/female)	7/11	6/11	5/7
ASS	12.72±2.63	7.59±2.18	0
FEV_1%	79.29±20.43	85.31±15.17	94.49±9.34
FEV_1/FVC%	71.35±9.65	90.12±25.30	96.13±11.40

All values are expressed as means ± standard deviation (M±SD). FEV_1% = predicted percentage of forced expiratory volume in the first second. FVC =forced vital capacity. ASS= asthma severity score. *represent no significant difference between the three groups .

and <4.5%, respectively. All samples were measured in duplicate.

Statistics

All data were expressed as means ± standard deviation (SD). Differences in the clinical characteristics and immunological data for the three groups were analyzed by one-way analysis of variance. Correlations analysis used the Spearman nonparametric test. The difference was considered to be significant at p <0.05. All analyses were tested using SPSS statistical software (version 17.0).

Results

Characteristics of the subjects

18 patients with allergic asthma, 17 controlled asthma patients and 12 healthy individuals were recruited for the study. The characteristics of all subjects are shown in Table 1. The FEV_1% in patients with allergic asthma was significantly lower than that in healthy controls (p <0.05), but there was no significant difference between the controlled asthma subject and healthy controls (p >0.05). The FEV_1/FVC% for allergic asthma sufferers was lower than those for the other two groups (p <0.05) and there were no significant difference between the controlled asthma and the healthy control groups. Patients with allergic asthma had higher ASS than those with controlled asthma and healthy controls (p <0.01). The ASS of patients with allergic asthma had a negative correlation with FEV_1 % (r =-0.75, p <0.05), which declined significantly with the severity of the disease. (Figure 1.)

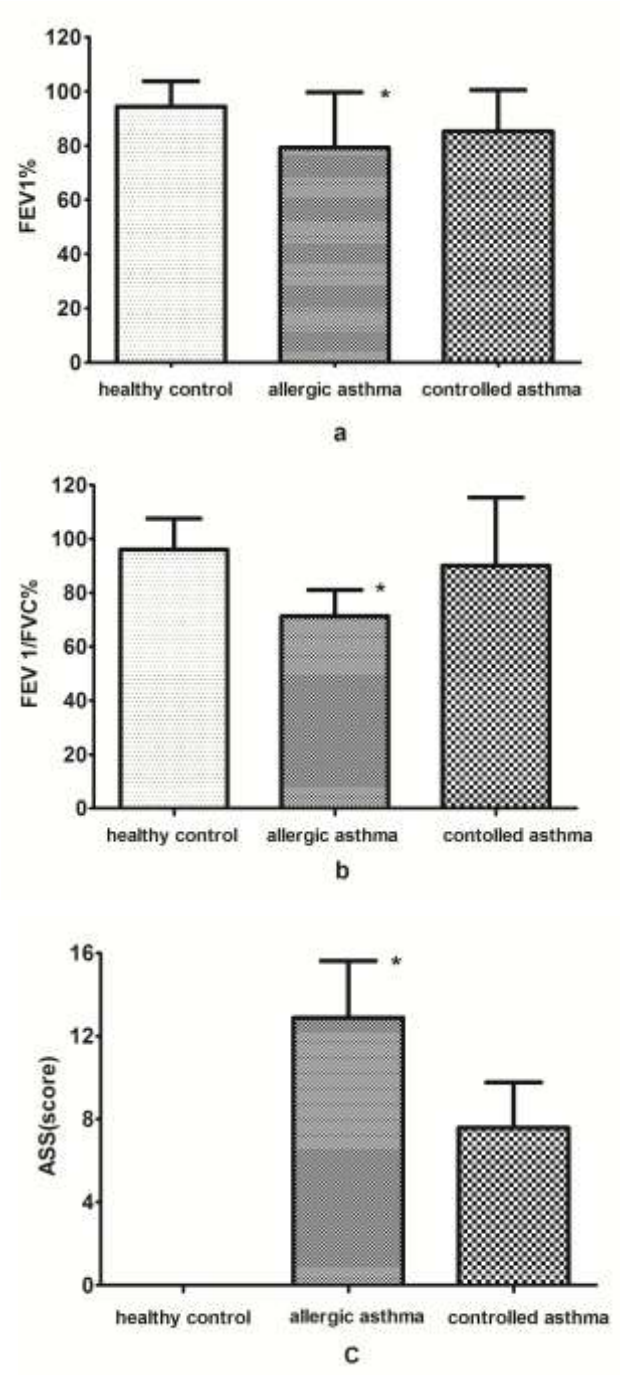

Figure 1. The differences in FEV_1%, FEV_1 /FVC% and ASS of healthy controls, allergic asthma cases, controlled asthma subjects. Statistical results were assessed by the S-N-K(S) test. The columns represent mean values ± SD. * p <0.05 compared with healthy control or controlled asthma (**a-c**).

Expression of IL -22 proteins in plasma

The levels of cytokine IL-22 in plasma were determined by IL-22 ELISA kits. Compared with those for controlled asthma subjects and healthy controls, the expression of IL -22 proteins in the plasma of allergic asthma sufferers was elevated significantly (p <0.01). The expression of IL -22 proteins in the plasma of controlled asthma subjects was slightly higher than that in healthy control but there was no significant difference. (Figure 2a.)

Expression of AhR mRNA in PBMCs

We detected the AhR mRNA levels in PBMCs using real-time PCR technology. As shown in Figure 2 b., the level of AhR mRNA was significantly increased in activated PBMCs in allergic asthma sufferers, as compared with controlled asthma subjects and healthy controls (p <0.05). However, the AhR mRNA expression was only slightly higher in controlled asthma subjects than that in healthy controls, and there was no significant difference between these groups.

Correlation between IL-22 concentrations or AhR mRNA level and FEV $_1$ %, FEV$_1$/FVC% or ASS of allergic asthma

We found that there was an up-regulation of AhR mRNA in PBMCs and IL-22 protein in the plasma from patients with allergic asthma. However, we surprisingly found that there was no correlation between AhR and IL-22 in patients with allergic asthma (p >0.05) (Figure 3a.). Correlation analyses revealed that the concentration of IL-22 in plasma correlated with the FEV_1%, FEV_1 /FVC% and ASS of patients with allergic asthma (r =-0.51, r =-0.47 and r =0.71 respectively) (Figure 3b-d.). We found there was no correlation between level of AhR mRNA in PBMCs and FEV_1%, FEV_1 /FVC% or ASS of patients with allergic asthma.

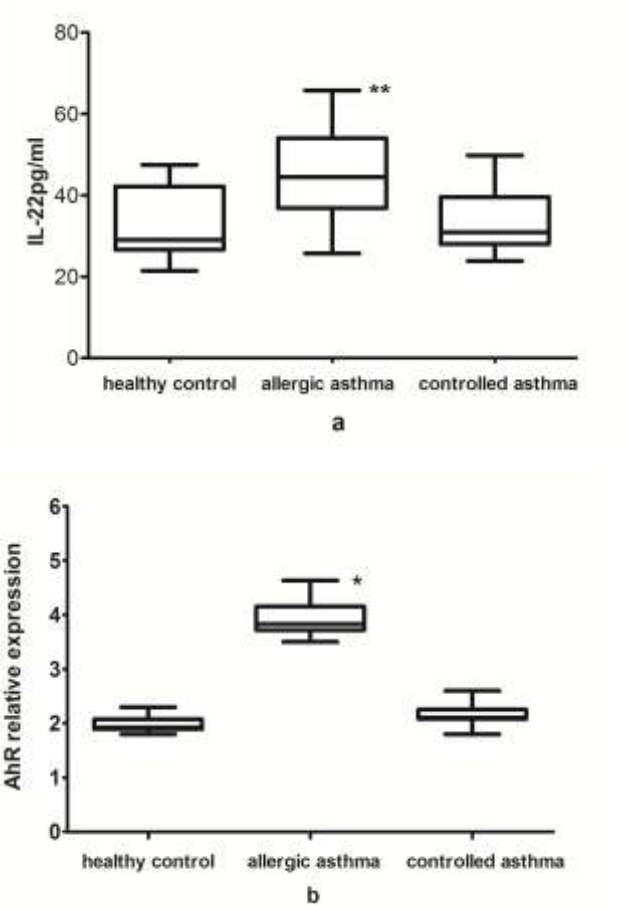

Figure 2. (**a**) Plasma concentrations of IL-22 protein from healthy controls, allergic asthma sufferers and controlled asthma cases. ** p <0.01 compared with healthy control or controlled asthma respectively. (**b**) Expressions of AhR mRNA in PBMCs from the three groups. Parallel box plots comparing AhR mRNA levels normalized to β-actin housekeeping gene presented as medians and ranges in healthy controls, allergic asthma sufferers and controlled asthma cases. * p <0.05 compared with healthy control or controlled asthma respectively.

Discussion

In our study, we demonstrated that there were significant increases in IL-22 protein in the plasma from patients with allergic asthma. The results were consistent with another study which had shown that T_H17 cells and IL-22 protein were increased in patients with allergic asthma[2]. High expression of IL-22 in plasma in allergic asthmatics may be derived from the increased number of circulating T_H17 cells, which are the main source of IL-22 in vivo. IL-22 is also derived from other activated T cells, such as Tc-cell subsets,γδT cells, natural killer (NK), and NKT cells[20].

The effects of IL-22 in the patient with allergic asthma are still unknown. Non-immune cells in lung organ were the most important target cells for IL-22 because they expressed IL-22R1 and IL-10R2[21]. IL-22 could induce antimicrobial proteins (AMPs) and up-regulate the expression of lipocalin 2 in the lungs as a defense mechanism against bacterial infection[22,23]. Additionally, in allergic mice models, IL-22 could down-regulate eosinophils and neutrophils, while the neutralization of IL-22 antibodies could augment the allergic response[11]. The mechanism might be that the expression of IL-22 was induced by allergic stimulation together with IL-23 and was controlled by an IL-4Rαsignal[11].

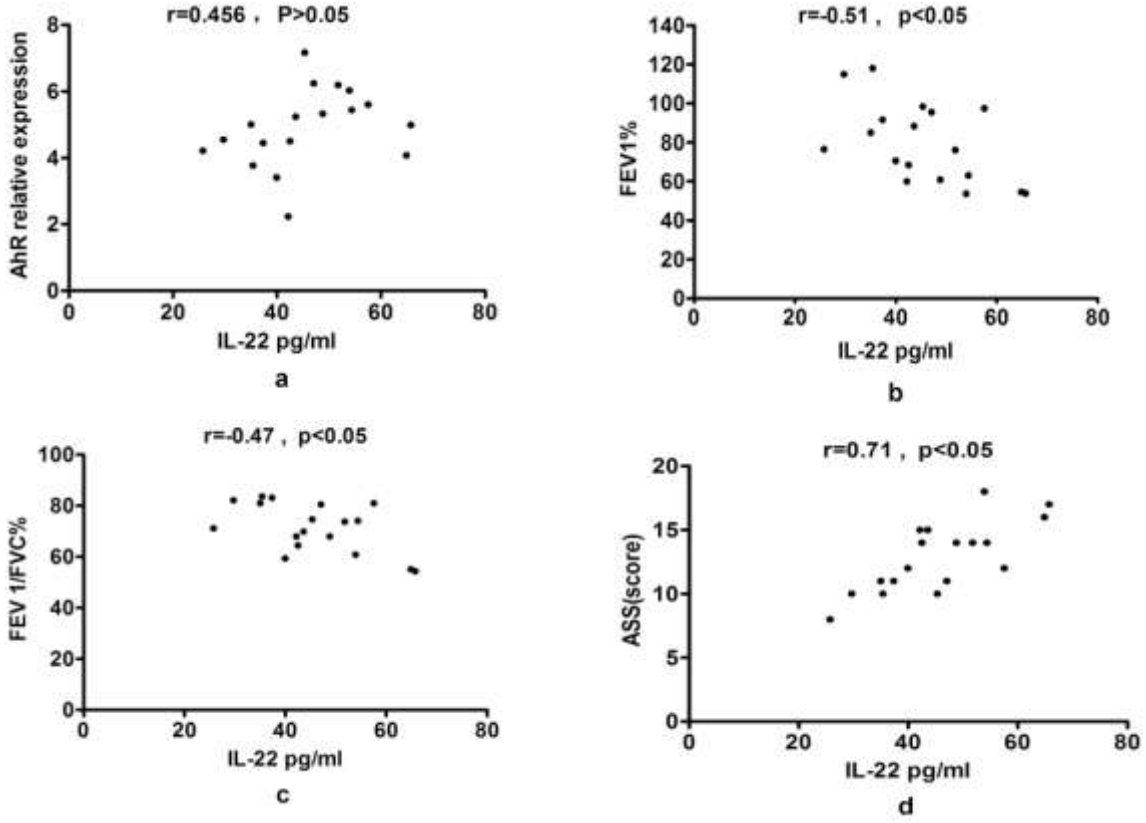

Figure 3. Concentration of IL-22 protein in plasma correlated with AhR, FEV_1%, FEV_1% /FVC, ASS and relative expression for patients with allergic asthma respectively (**a-d**). Statistical analysis was carried out using Spearman's rank correlation test.

IL-22 has modest inhibitory effect on IL-4 production derived from T_H2 cells, which play potential therapeutic roles in eosinophil-dominant asthma[24].

Allergic asthma is characterized by chronic airway inflammation and airway hyper-responsiveness. A variety of cytokines such as IL-17 and eotaxin are involved in the local airway inflammation, airway remodeling and reduced lung function of patients with allergic asthma. The mechanism may be that cytokines stimulate human bronchial fibroblasts, airway epithelial cells and airway smooth muscle cells to produce various pro-inflammatory cytokines or chemokines, such as IL-6, IL-8, growth-related oncogene-α（Gro-α）, that promote contraction of airway smooth muscle and increased resistance to airflow[25-27]. We also found that the level of IL-22 in plasma was highly correlated with the level of FEV_1%, FEV_1 /FVC % and ASS of patients with allergic asthma. This suggests that IL-22 might be a systemic reflection of airway local inflammation in allergic asthmatics and might have a potential role as an index to help us assess the degree of asthma. It also may have a similar role to some of above cytokines that result in airway inflammation and lead to decreased lung function of patients. Though Schnyder et al. reported that IL-22 is a negative regulator of the allergic response, Zhao et al. found an increase of IL-22 level in plasma in patients with allergic asthma[11, 2]. We thought that there may be some difference between role of IL-22 in airway inflammation in patients with allergic asthma and in allergic mice models. Whether IL-22 plays protective role or pathogenic role in allergic asthma still is not clear. Thus we still need to further discuss the role of IL-22 in future asthma models or similar animal models.

AhR is activated by AhR agonists such as TCDD, resulting in suppression of immune responses. In this study, we also found that the expression of AhR mRNA was markedly increased in the patients of allergic asthma. AhR mRNA levels

for patients with controlled asthma were decreased. This may because T_H17 cells were inhibited by treatment, such as using glucocorticoids[28]. Kimura et al. found that AhR regulated differentiation of T_H17 cells by STAT1 and enhanced the expression of IL-22 through Notch signaling[29]. However, surprisingly in our study the level of AhR mRNA was not related to the level of IL-22. This result may be explained as follows: the retinoid –related orphan receptor C (RORC) and AhR were similarly involved in the regulation of IL-22 production[30]; the PBMCs consisted of many immune cells in which Treg cells also could express small amount AhR; not all AhR transcripts did translate the AhR protein[30]; there are different effects on Treg and TH17 cells caused by different ligand- activated AhR. For example, AhR activation by TCDD could induce Treg cells to suppress the function of immune system and FICZ could boost T_H17 cells differentiation and then prompt the development of autoimmune diseases[31]. Additionally, AhR agonist could activate the AhR signaling pathways to modulate the T_H1/ T_H2 balance to T_H2 inducing the development of allergic diseases[32]. We did not find that the AhR mRNA was correlated to clinical indices of patients with allergic asthma, probably because the level was a comprehensive result affected by various factors.

In summary, we found that both the elevated AhR mRNA in PBMCs and IL-22 protein in plasma were important features of allergic asthma and IL-22 may have an indirect correlation with the severity of allergic asthma, may be involved in the pathogenesis of allergic asthma and may become a novel auxiliary diagnostic and therapy target for allergic asthma. Further studies of the molecular mechanism of AhR and IL-22 in allergic asthma may be necessary.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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