

# Decreased concentration of IL-35 in plasma of patients with asthma and COPD

Chen Chen, Yanhan Deng, Huilong Chen, Xiaojie Wu, Sheng Cheng, Yongjian Xu, Weining Xiong and Jungang Xie

## Summary

**Background:** IL-35 has been found to be involved in many inflammatory diseases in humans but its role in asthma and chronic obstructive pulmonary disease (COPD) is not clear. The plasma level of IL-35 in patients with asthma and COPD needs to be measured.

**Objective:** The aim of this study was to examine the plasma concentrations of IL-35 in newly diagnosed asthmatic and COPD patients and control subjects and investigate correlations of lung function, age, sex, smoking history with the levels of IL-35 in plasma in these diseases.

**Methods:** Blood samples were collected from patients with newly diagnosed asthma (44, 12 males, aged  $33.75 \pm 8.94$ ), newly diagnosed COPD (36, 36 males, aged  $68.03 \pm 8.94$ ), and healthy control groups (23, 9 males, aged  $30.06 \pm 7.50$ ). We determined the IL-35 levels in plasma by enzyme-linked immunosorbent assay.

**Result:** The median and the range of values for IL-35 were 118.55 pg/ml (range 74.43~1767.22 pg/ml) in patients with asthma, 136.09 pg/ml (range 62.54~697.49 pg/ml) in patients with COPD and 275.86 pg/ml (range 26.11~1766.20 pg/ml) in control subjects. The levels of IL-35 in plasma showed a positive correlation with FEV1% and FVC% in asthmatic patients whose plasma IL-35 values were over 150 pg/ml. A positive correlation was also found between plasma IL-35 and FVC% in COPD patients whose plasma IL-35 values were over 150 pg/ml.

**Conclusions:** These findings suggest that IL-35 may very probably be involved in the Th2 and Th17 mediated inflammation process of asthma and COPD. Its role in the mechanisms of COPD and asthma in human beings, as well as its therapeutic value in these diseases, need further investigation. (*Asian Pac J Allergy Immunol* 2014;32:211-7)

**Keywords:** IL-35, asthma, COPD, cytokine, pulmonary function

## Introduction

Asthma and COPD are both well-known airway diseases, which are characterized by chronic inflammation of the respiratory tract. According to GINA, as many as 300 million people worldwide suffer from asthma and the burden of COPD in China varies from 5% to 13% in different provinces/cities.<sup>1</sup>

Although these two diseases share some similarities in terms of clinical manifestations, the differences in patho-physiology are marked. The immunological mechanisms underlying asthma are heterogeneous. Recent studies have reported that Th2 plays a central role in orchestration of the inflammatory process in asthma. By releasing cytokines, Th2 cells recruit B cells, eosinophils and mast-cell into the network of the airway inflammatory reaction. In more severe asthma, significant infiltration of neutrophils has been observed<sup>2</sup> and Th17 cells are thought to be the main mediator of the recruitment of neutrophils in severe asthma. Cytokines released by Th17 cells make airway epithelial cells release chemokines to attract neutrophils. Also, another subtype of CD4<sup>+</sup> T cells, the regulatory T cells, have been proved to play an important role in asthma.<sup>3-5</sup> Regulatory T cells are thought to have an anti-inflammatory activity by suppressing Th2 responses. In COPD, CD8<sup>+</sup> T cells play a dominant role in the pathogenesis and the local infiltrations in COPD mainly consist of neutrophils.<sup>6,7</sup>

\*Chen Chen and Yanhan Deng have contributed equally to this work.

From Department of Respiratory and Critical Care Medicine, Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Corresponding author: Jungang Xie

E-mail: [xiejgg@hotmail.com](mailto:xiejgg@hotmail.com)

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IL-35 is the newest member of the IL-12 family. In its murine counterpart, IL-35 has been shown to be expressed preferentially by Foxp3<sup>+</sup> Treg cells but not resting or active effector T cells.<sup>8,9</sup> It is a novel cytokine that suppresses the immune response by inducing the proliferation of regulatory T cells and inhibiting the differentiation of Th17 cells.<sup>7</sup> Recently, a study has shown that IL-35 can limit airway inflammation and IgE production in a dust mite allergen-specific mouse models.<sup>10,11</sup> In several diseases in humans, such as chronic hepatitis B virus infection and coronary artery diseases, IL-35 has also been reported to be involved in the pathogenesis of the inflammatory process.<sup>12,13</sup> The mRNA expression of Ebi3 and p35, the two subunits of IL-35, can be detected in CD4<sup>+</sup> T cells from the peripheral blood of chronic hepatitis B patients, while there is no Ebi3 expression in the CD4<sup>+</sup> T cells of healthy donors.<sup>14</sup> A decreased plasma level of IL-35 has been detected in patients with coronary artery diseases.<sup>15</sup>

However, to the best of our knowledge, the plasma concentration of IL-35 has not yet been systematically evaluated in patients with asthma and COPD. In this study, we measured the plasma concentrations of IL-35 from patients with asthma or COPD and healthy control subjects by ELISA. We also recorded parameters of lung function to look into the relationship between IL-35 concentrations and the severity of airway disease.

## Methods

### *Asthmatic patients, COPD patients, control subjects*

Forty-four Chinese patients (aged 34±9 years, ranged:15-52) newly diagnosed with asthma were recruited by the Department of Respiratory and Critical Care Medicine, Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology. The diagnosis was based on the asthma guidelines proposed by the American Thoracic Society. None of the asthmatic patients recruited had undergone any ICS therapy before having their blood tested and undergoing lung function tests.

Twenty-three sex-matched healthy Chinese volunteers (aged 30.06±7.5 years, ranged: 21-47) were recruited as normal controls. All the control subjects were screened by a questionnaire and a simple physical examination to make sure that they had no abnormal signs present and no history of asthma or COPD or other autoimmune diseases.

The number of newly diagnosed chronic obstructive pulmonary disease (COPD) patients in this study was 36, and the diagnosis was based on GOLD. Every COPD patient involved in this study was free from other inflammatory comorbidities, for instance exacerbations, in the recent past.

The terms of written consent to participate in this study related to the concerns of all those involved. The collection of blood samples and the related assays were approved by the Ethical Committee of the Medical Faculty of Tongji Medical College.

### *Sample collection*

We used ethylenediamine tetraacetic acid (EDTA) as an anticoagulant to collect plasma and then centrifuged the blood samples at 4000 rpm at 4°C for 15 minutes almost immediately. The samples were stored in microfuge tubes at -80°C until the measurements were taken.

### *IL-35 measurement*

Plasma IL-35 was quantified by using a commercial human Interleukin-35 (IL-35) ELISA kit (Bio-Swamp, Catalog NumberHM10199) using the manufacturer's protocol. All samples were assayed in duplicate. The mean concentration was determined for each sample. All the experiments were done by the same researchers in the same laboratory.

### *Statistical analysis*

All the data analysis was carried out with The Statistical Package for Social Sciences (SPSS) 13.0 version. The results were presented as mean±SD unless otherwise specified. The variations in the demographic data of asthmatic patients and COPD patients (Table 1) were compared with control subjects respectively, using an independent t-test (comparison of IL-35 concentration used non-parametric statistical analysis). The analysis of the difference in plasma IL-35 concentration among the above-mentioned 3 study groups (Figure 1) was done using ANOVA, followed by the Turdey post hoc test. Coefficients for plasma IL-35 concentrations and different pulmonary function indicators (Table 2-3), as well as other possible correlated factors (senior age, smoking history, gender), were determined using Spearman's rank correlation test. In order to observe the relationship between plasma IL-35 concentrations and these pulmonary function indicators, we used Curve Estimation, where all the models were settled as exponential. Any value of *P* <0.05 was considered to be statistically significant.



**Table 1.** Characteristics of the study groups

		asthma (n=44)	control (n=23)	COPD (n=36)
age(years)	mean±SD	33.75±8.94	30.06±7.50	68.03±8.94*
	range	15~52	21~47	48~88
sex(% male)	ratio	27.27%	39.13%	100%*
smoking (packs/year)	ratio	13.33%	-	100%
	mean±SD	1.82±5.64	-	43.89±22.23
	range	0~29	-	2.6~102
FEV1/FVC	mean±SD	0.74±0.12**	0.87±0.06	0.59±0.03*
	range	0.39~0.96	0.75~1.00	0.55~0.71
FEV1%	mean±SD	82.70±22.25**	103.61±11.39	71.72±26.17*
	range	19.3~117	78~124	30.0~138.0
FEV1(L)	mean±SD	2.55±0.76**	3.21±0.60	1.37±0.61*
	range	0.59~5.08	2.42~4.27	0.50~2.64
FVC%	mean±SD	95.94±17.94**	106.00±13.78	93.93±24.14
	range	43.1~142	79~127	52.0~139.0
FVC(L)	mean±SD	3.44±0.87	3.74±0.82	2.51±0.81*
	range	1.53~5.39	2.47~5.76	1.26~4.02
PEF(L)	mean±SD	5.35±1.87**	7.56±2.26	-
	range	0.34~9.41	4.32~14.40	-
IL-35(pg/mL) <sup>#</sup>	median	118.55**	275.86	136.09*
	range	74.43~1767.22	26.11~1766.20	62.54~697.49

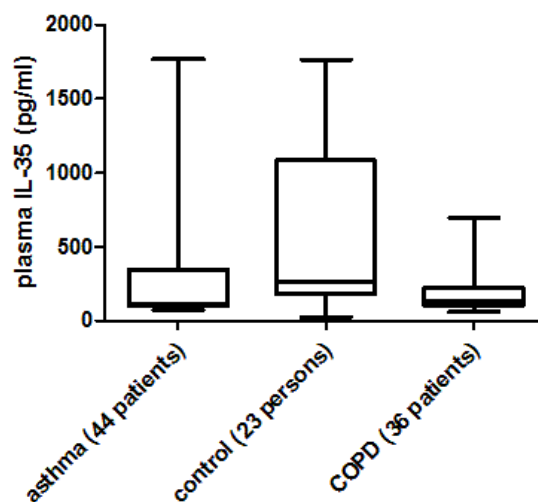
\*\*: $p < 0.05$  asthma vs. control; \*:  $p < 0.05$  COPD vs. control; <sup>#</sup>: using non-parametric statistical analysis

## Results

Altogether 103 eligible blood samples were evaluated during the study, including 44 from asthmatic patients, 23 from control subjects and 36 from COPD patients.

### Characterization information of subjects

There were no significant differences in age and gender between the asthmatic patients and control subjects, meanwhile the COPD patients were significantly ( $p = 0.000$ ) older than the healthy controls. Also, all the COPD patients involved in this study were male, which was significantly ( $p = 0.000$ ) different from the control subjects. Patients in the COPD group had significant ( $p = 0.000$ ) greater history of smoking than the asthmatic group. In this study, asthmatic patients were obviously worse in terms of pulmonary function, including FEV1/FVC ( $p = 0.000$ ), FEV1 ( $p = 0.000$ ), FEV1% ( $p = 0.000$ ), FVC ( $p = 0.030$ ) and PEF ( $p = 0.000$ ), than the control subjects, while the FVC level showed no difference ( $p = 0.175$ ) between the other two groups. FEV1/FVC, FEV1, FEV1% and FVC% in the COPD group were significantly ( $p = 0.000$ ) lower as compared with those in the healthy control group, but the level of FVC did not show a



**Figure 1.** Plasma IL-35 levels in study groups.

difference ( $p = 0.065$ ). (Table 1) The plasma IL-35 concentrations are discussed below and can also be seen in Table 1.

### Plasma IL-35 concentration

In asthmatic patients, the median concentration of plasma IL-35 was 118.55 pg/ml, with the highest value 1767.22 and lowest 74.43 pg/ml.



**Table 2.** Spearman's rank correlation coefficients

	all asthmatic patients (n=44)		asthmatic patients with IL-35>150 pg/ml (n=18)		all COPD patients (n=36)		COPD patients with IL-35>150 pg/ml (n=15)	
	Spearman Correlation	Sig.	Spearman Correlation	Sig.	Spearman Correlation	Sig.	Spearman Correlation	Sig.
age	0.048	0.755	--	--	-0.342	0.041*	--	--
FEV1%	0.100	0.503	0.639	0.004*	-0.007	0.973	0.565	0.089
FEV1/L	0.18	0.238	0.461	0.054	-0.04	0.983	0.379	0.164
FVC%	0.194	0.201	0.482	0.043*	0.002	0.993	0.648	0.043*
FVC/L	0.208	0.169	0.198	0.430	0.063	0.715	0.416	0.123
PEF/L	0.106	0.489	0.379	0.121	--	--	--	--
FEV1/FVC	0.055	0.721	0.542	0.020*	-0.182	0.289	0.302	0.273

\*: correlation is significant at 0.05 level (2-tailed)

In the control group, the median value of plasma IL-35 concentration was 275.86 pg/ml, with a range of 26.11~1766.20 pg/ml.

The median concentration of plasma IL-35 in the COPD group was 136.09 pg/ml, ranging from 62.54 to 697.49 pg/ml.

Further analysis showed that the values of plasma IL-35 concentration in asthmatic ( $p=0.001$ ) and COPD ( $p=0.000$ ) group were lower than those of control subjects. More than half of the patients in the asthmatic and COPD groups had plasma IL-35 values lower than 150pg/ml, while in the control group it was only 13.04%. Compared with asthmatic and COPD groups, there were more cases with plasma IL-35 values higher than 1000 pg/ml in the healthy control group, while only 1 case in COPD group had a plasma IL-35 value over 500 pg/ml.

Asthmatic and COPD patients tended to have less IL-35 secretion compared with healthy controls, which suggests that impairment of IL-35 production may be connected with a higher liability to develop airway inflammation.

Also, a negative correlation between age and plasma IL-35 was observed in the COPD group ( $p=0.041$ ). Older patients tended to have lower plasma IL-35 values than the younger ones (Table 2). Apart from this, no significant correlation was found between plasma IL-35 and other demographic characteristics (gender, smoking history).

#### **Correlation between plasma IL-35 concentration and pulmonary function**

Plasma IL-35 concentrations were not correlated with any lung function indicators in the asthmatic patients, nor in the members of the COPD group. If

we look at all the cases with a relatively high concentration of IL-35 (over 150 pg/ml), correlations can be found (Table 2). Plasma IL-35 was positively correlated with increased FEV1% ( $p=0.004$ ), FEV1/FVC ( $p=0.020$ ) and FVC% ( $p=0.043$ ) in asthmatic patients, and FVC% ( $p=0.043$ ) in COPD group. Exponential models could better describe the correlation between elevated plasma IL-35 and lung function in asthmatic cases (Table 3, Figure 2: C, D).

Cases with plasma IL-35 below 150pg/ml showed no correlation between plasma IL-35 and lung function indicators.

#### **Discussion**

IL-35 works as an inflammation inhibitor in several autoimmune diseases, such as collagen-induced arthritis, T-cell-dependent colitis,<sup>16,17</sup> but it does not show the ability to limit the inflammation of Lyme Arthritis.<sup>18</sup> Its effect in asthma and COPD remain to be investigated.

Our data in this report showed that the plasma concentrations of IL-35 were detectable in all the three groups. Although the study by Collison LW shows that the expression of IL-35 is not constitutive in tissues,<sup>8,19</sup> two viruses have been reported to induce the secretion of IL-35, one of which is Human rhinoviruses<sup>12,20</sup> and the others Hepatitis B.<sup>14,21</sup> Vascular endothelial cells, smooth muscle cells and monocytes also have the potential to express IL-35 after activation with proinflammatory cytokines and lipopolysaccharides.<sup>22,23</sup> This suggests that the background secretion of IL-35 into the circulation might be contributed by other kinds of cells, not only by Treg cells.



**Table 3.** Regression analysis of IL-35\* with different pulmonary function indicators\*\* in asthma patients with IL-35>150 pg/mL (n=18)

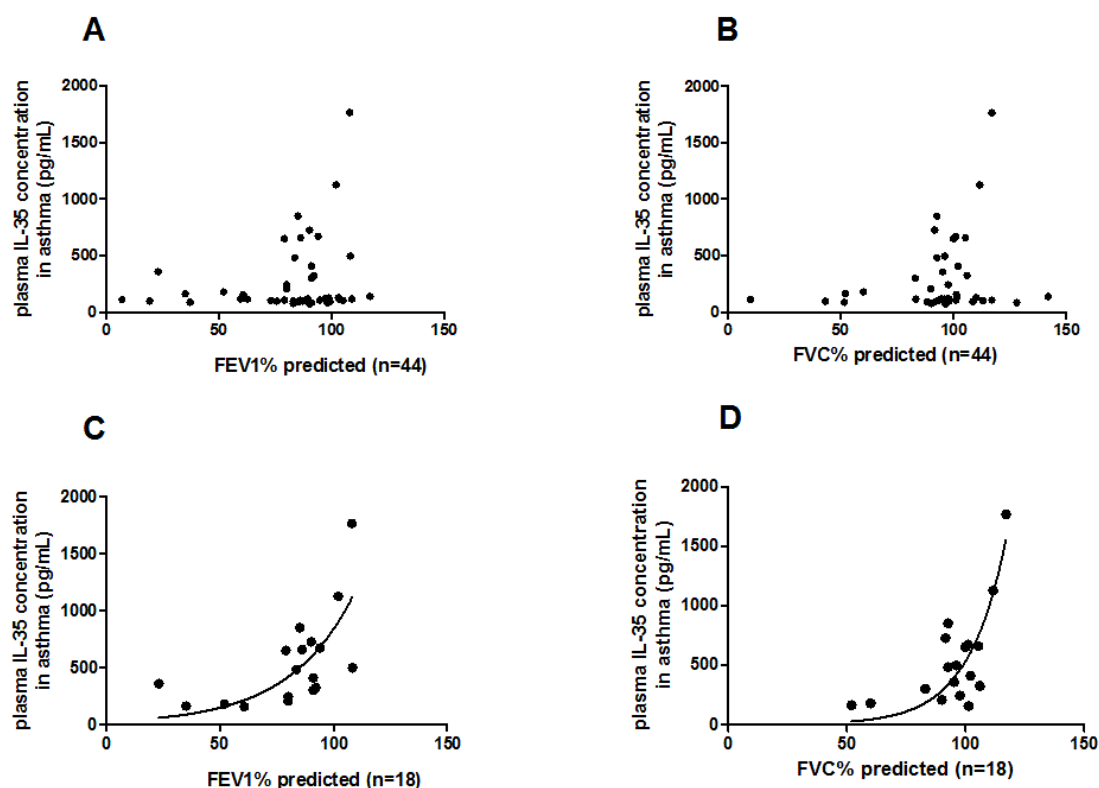
	R <sup>#</sup>	R square <sup>#</sup>	unstandardized coefficients		standardized	t value	p value
			B	standard error	coefficients		
					Beta		
FEV1%	0.632	0.399	0.019	0.006	0.632	3.258	0.005
FVC%	0.646	0.417	0.028	0.008	0.646	3.382	0.004

\*:IL-35 concentration as dependent variable; \*\*:pulmonary function indicators as independent variables.

<sup>#</sup>:predictor contains constant.

Our findings also showed that the IL-35 plasma levels were higher in control subjects than that in asthmatic patients and these differences were statistically significant. Besides, nearly half of the healthy subjects had levels higher than 300pg/ml. The low concentrations in the asthmatic and COPD patients is probably the low background secretion of IL-35 that had been found before. One possible reason might be that there were all kinds of stimulators or allergens in the environment in daily life, therefore the immune system might be activated every now and then and this may explain why so many control subjects' plasma IL-35 values were

elevated. When the anti-allergy mechanism couldn't function well, such as in the presence of impairment of IL-35 production, which could be described as low plasma IL-35 concentration stimulation, airway-inflammation, asthma and COPD, or other type of immune disorder might be more likely to take place. IL-35 production has been found to reverse IL-17 -dependent allergic airways disease.<sup>24,25</sup> Therefore, lower IL-35 level might indicate less suppression of an IL-17-dependent allergic process. IL-17 is mainly secreted by Th17 cells, which are thought to be the central mediator of the neutrophils recruitment in severe asthma.<sup>2</sup> A recent study has shown that Th17

**Figure 2.** Correlation IL-35 concentration and lung function in asthma

cells increase significantly in the asthmatic process.<sup>26</sup> This might result from reduced IL-35 levels in the circulation. Apart from Th17 cells, asthma is also strongly correlated with Th2 cells. Th2 development can be blocked by IL-35 as a result of suppression of IL-4 and the transcription factor GATA-3, thereby limiting the proliferation of Th2 cells (V.Chaturvedi and D.A.A.V., unpublished data).<sup>27</sup> It could be suggested from these pathophysiological findings that insufficient expression of IL-35 may partly cause the development of asthma.

Those few asthmatic patients who had higher IL-35 levels (over 1000pg/ml) showed better airway conditions in their lung function examinations. Since asthma is a disease characterized by reversible airflow limitation, this finding may indicate that higher IL-35 levels in circulation may have a positive effect on suppressing airway inflammation and reversing airflow limitation.

Our data also indicated that the plasma levels of IL-35 in COPD were the lowest among the three groups and the peak numbers for COPD were also lower. Unlike in asthma, CD8<sup>+</sup> T cells are the common cells in the airways and lung parenchyma of patients with COPD, rather than CD4<sup>+</sup> T cells.<sup>28,29</sup> However, as what we know now, IL-35 is secreted mainly by CD4<sup>+</sup>Foxp3<sup>+</sup> regulatory T-cell. This may partly explain the reason why the expression of IL-35 was limited in COPD patients, as compared with control subjects. Besides that, by contrast with asthmatic patients and control subjects, patients in COPD group were all male and reported greater numbers of smoking pack-years than those in the other groups. COPD patients were significantly older than the individuals in the other two groups. Although we found no significant correlation between IL-35 and demographic characteristics (such as gender, age and smoking) in this study, we still can't exclude these demographic factors because of the limited size of the sample. Therefore, a larger sample study is needed to validate importance of IL-35.

In conclusion, our finding of decreased circulating IL-35 levels in asthma and COPD patients supports the hypothesis that IL-35 may be involved in the pathogenesis of these two diseases. Further investigations are necessary to determine the sites, mechanisms, and the consequences of IL-35 in these two inflammatory disorders.

In recent years, novel therapies such as anti-IL5, anti-IL13 and tyrosine kinase inhibitors have gradually come into clinical use.<sup>30</sup> Whether IL-35

has a potential therapeutic value for asthma or COPD needs further investigation.

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### Conflicts of interest

None of the authors have a conflict of interest to declare in relation to this work.

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