

SOX40L: An Important Inflammatory Mediator in Adult Bronchial Asthma

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Abstract

Introduction: The role of soluble OX40 ligand (sOX40L) in adult bronchial asthma is unclear. This study aims to determine the serum concentrations of sOX40L in adult patients with bronchial asthma, and discussed its relationship with pulmonary function. **Materials and Methods:** We measured the pulmonary function using the spirometer and detected the serum concentrations of sOX40L by enzyme linked immunosorbent assay (ELISA) in 19 healthy persons in the control group, 58 acute asthmatic adult patients who were grouped according to their disease severity: 18 mild grade, 24 moderate grade, 16 severe grade, and 24 persons in a stable asthmatic group. **Results:** The serum concentrations of sOX40L in asthmatic adult patients (6.80 ± 4.95 ng/L) were distinctly higher than those in the control group (3.98 ± 2.83 ng/L, $P < 0.05$), and they were negatively correlated with pulmonary function indexes (FEV1%, FVC%, FEV1/FVC) ($r = -0.754$, $P < 0.01$, $r = -0.557$, $P < 0.01$, $r = -0.457$, $P < 0.01$, respectively). Moreover, the serum concentrations of sOX40L showed obvious differences among control, mild, moderate, and severe groups (3.98 ± 2.83 , 4.87 ± 1.89 , 6.97 ± 5.91 , 8.71 ± 5.18 ng/L, respectively; $P < 0.01$). The concentrations of sOX40L decreased to the same extent as the control group after therapeutic treatments were provided to the asthmatic adult patients. **Conclusion:** The concentrations of sOX40L were found to be high in adult asthmatic patients and were associated with the severity of the disease. Therefore, sOX40L could be a potential inflammatory mediator in the pathogenesis of asthma.

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Introduction

Bronchial asthma is a kind of chronic airway inflammatory disease which is associated with the infiltration of eosinophils, mastocytes, and T lymphocytes, and the release of several inflammatory mediators.^{1,2} These inflammatory mediators play an important role in the pathogenesis of asthma. Recent studies suggest that many co-stimulatory molecules such as CD30, tumour necrosis factor receptor (TNFR), CD40, CD40L, B7-H3 etc exist in membrane forms and soluble forms.³⁻⁶ In many diseases, these soluble molecules are highly important in disease diagnosis, severity assessment, clinical staging, and disease prognosis.

As a vital co-stimulatory signal molecule in the TNFR/TNF family,⁷ OX40L is also found to exist in membrane forms

and soluble forms.⁸ Studies show that the concentrations of soluble OX40L (sOX40L) increase in many diseases.^{9,10} Recently, Ezzat MH et al¹¹ found that sOX40L levels were significantly higher in asthmatic children than those in the control group. The up-regulation of sOX40L correlated with the severity of the childhood bronchial asthma, whereas few studies have reported the role of sOX40L in adult bronchial asthma. Does sOX40L play a different role in adult and children patients? In our study, we measured the serum concentrations of sOX40L by means of enzyme linked immunosorbent assay (ELISA) in adult asthmatic subjects, and preliminarily examined its role in the pathogenesis of adult bronchial asthma.

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Materials and Methods

Study Population

A total of 101 subjects were included in this study. The control group consisted of 19 healthy volunteers without obvious malfunctions in the heart, liver, kidneys, or lungs. Subjects with a past, current, or family history of allergic diseases and autoimmune disorders were excluded. The acute asthmatic patient group consisted of 58 adult asthmatic patients. The stable asthmatic group consisted of 24 adult asthmatic patients in stable stage. The clinical features are summarised in Table 1. All the patients fulfilled the criteria for the Global Initiative for Asthma's (GINA) guidelines.¹² The study was approved by the Ethics Committee of the First Affiliated Hospital of Soochow University and informed consent was obtained from all participants.

Table 1. Main Clinical Characteristics of the Study Population

| Characteristic | Control | Stable | Acute | P value |
|------------------------------|-------------|-------------|------------|---------|
| Gender (Male : Female) | 9:10 | 13:11 | 28:30 | 0.89 |
| Age in years (mean ± SD) | 37.1 ± 10.7 | 35.0 ± 10.8 | 35.8 ± 9.7 | 0.75 |
| Smoking status (Yes : No) | 5:14 | 4:20 | 8:50 | 0.39 |

The medical history of the stable asthmatic patients group was as follows: 10 patients (41.7%) were maintained on inhaled glucocorticosteroids (ICS) + long-acting inhaled β 2-agonist (LABA), 6 (25.0%) were maintained on ICS, 3 (12.5%) were maintained on sustained-release theophylline, 2 (8.3%) were maintained on ICS + LABA + sustained-release theophylline, 2 (8.3%) were maintained on leukotriene modifiers, 1 (4.2%) was maintained on ICS + LABA + leukotriene modifiers.

Obtaining Blood Samples and Detection of sOX40L

For healthy volunteers and stable asthmatic patients, blood was taken early in the morning of the appointed date after overnight fasting; for the acute asthmatic patients, blood was taken before treatment. Peripheral venous blood samples from each candidate were drawn into pyrogen-free blood

collection tubes without additives. Three milliliters of blood was drawn from each candidate, kept immediately at 4°C and allowed to clot for 1 hour before centrifugation. We centrifuged the serum at 2000 r/min for 10 minutes, then kept the separated serum sterilised at the temperature of -70°C prior to use. Samples were thawed only once. We measured the serum concentrations of sOX40L using ELISA kits (Cusabio, China) according to the instructions of the kit. The range of sOX40L detected was 0.8 ng/L to 15 ng/L.

Measuring of Pulmonary Function

All participants underwent pulmonary function tests using a spirometer (Jaeger, Germany). We calculated the forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) percentage against the predicated value (FEV1% and FVC%), and then we computed FEV1 percentage against FVC (FEV1/FVC).

Statistical Analysis

We analysed all acquired data using SPSS11.5 software, and the results were demonstrated in the form of mean standard deviation ($\bar{x} \pm s$). The differences among multiple groups were analysed by one-way ANOVA (post hoc LSD). The difference was judged as statistically significant when $P < 0.05$. Correlation analysis was conducted using the Spearman's rank correlation coefficient method.

Results

Relation between Serum Concentrations of sOX40L and Pulmonary Function Data

The pulmonary function indexes FEV1%, FVC%, FEV1/FVC and the serum concentrations of sOX40L differed in the control group, acute group and stable group. For the acute group, the pulmonary function indexes (FEV1%, FVC%, FEV1/FVC) were much lower and the serum concentrations of sOX40L were higher when compared to the control group. The above indexes in the stable group were similar to that in the control group (Table 2). Inflammatory factor serum sOX40L showed a significantly negative correlation with pulmonary function indexes (FEV1%, FVC%, FEV1/FVC) ($r = -0.754, P < 0.01$; $r = -0.557, P < 0.01$; $r = -0.457, P < 0.01$, respectively).

Table 2. Serum Concentrations of sOX40L and Pulmonary Function Data ($\bar{x} \pm s$)

| Group | No. | FEV1% (%) | FVC% (%) | FEV1/FVC (%) | sOX40L (ng/L) |
|---------|-----|---------------|---------------|---------------|---------------|
| Control | 19 | 97.55 ± 5.88 | 98.98 ± 5.96 | 79.24 ± 4.57 | 3.98 ± 2.83 |
| Acute | 58 | 63.98 ± 20.81 | 74.76 ± 18.41 | 62.74 ± 13.10 | 6.80 ± 4.95 |
| Stable | 24 | 94.67 ± 6.62 | 99.21 ± 5.23 | 77.22 ± 5.10 | 4.33 ± 3.32 |
| F value | | 47.16 | 34.84 | 26.79 | 4.69 |
| P value | | <0.01 | <0.01 | <0.01 | <0.05 |

Serum Concentrations of sOX40L and Pulmonary Function Data in Acute Asthmatic Patients

In accordance to disease severity, acute asthmatic patients group could be divided into mild grade (n = 18), moderate grade (n = 24), severe grade (n = 16), and respiratory arrest imminent grade.¹² The pulmonary function data FEV1%, FVC%, FEV1/FVC and serum concentrations of sOX40L among mild grade, moderate grade, and severe grade were shown in Table 3. Serum concentrations of sOX40L in moderate grade were found to be higher than those in mild grade. However, the serum concentrations for severe asthmatics were found to be about twice as those of mild asthmatics (Fig. 1). We also measured the concentrations of sOX40L in 3 respiratory arrest imminent asthmatic patients, whose serum concentrations of sOX40L were found to be higher than the norm, but because of the small number and the lack of pulmonary function data, these data were not included in the statistical analysis.

Discussion

Bronchial asthma is a kind of airway allergic inflammation

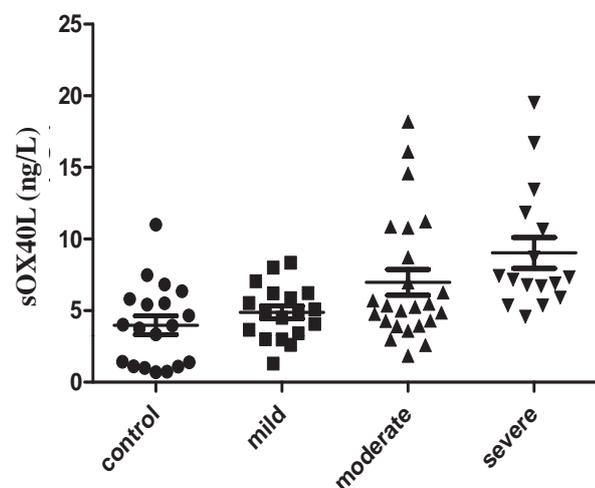


Fig. 1. Serum concentrations of sOX40L among mild grade, moderate grade, severe grade showed statistical significance ($P < 0.05$). The more serious the disease, the higher concentrations of sOX40L.

disease caused by several inflammatory cells as well as by mediators with complicated pathogenesis. Inflammatory cells and released mediators play an important role in the pathogenesis of asthma.^{13,14} Our study focused on the increased serum concentrations of sOX40L in adult asthmatic patients, which indicated the associations of the mediator with pulmonary function and disease severity. SOX40L may play a vital role in the pathogenesis of asthma.

Human OX40 Ligand (OX40L, gp34, CD252, TNFSF4 or CD134L) is a type II transmembrane glycoprotein that belongs to the TNF super-family. In 1991, Miura S et al¹⁵ cloned human OX40L. Human OX40L is mainly expressed on mature dendritic cells (DCs), active B cells, endothelial cells, and macrophages, as well as, some organs including the lungs, heart, skeletal muscle, and testicle, etc.^{16,17} Membrane type ligand OX40L interacts with membrane-type OX40 to mediate activation, quantity expansion, transportation, and life expansion of CD4⁺ T cells, as well as, to promote the formation of germinal centers and the maturation of DCs.¹⁸⁻²¹ The interactions of OX40/OX40L have been found to play a critical role in the development of many inflammatory and autoimmune diseases.^{22,23} Some studies^{24,25} found that many asthmatic responses were not induced in OX40L-deficient BALB/c mice. Administration of neutralizing anti-OX40L mAb in wild-type BALB/c mice also abrogated the induction of asthmatic responses. These results indicated that OX40L played an important role in the development of pathogenic Th2 cells in a murine model of asthma. However, significant research on the sOX40L molecule's function in disease appears to be rare. Qin W et al⁹ showed that the concentrations of sOX40L in patients with Henoch-Schonlein purpura were much higher than those in the control group. In addition, the authors discovered that the levels of sOX40L were obviously elevated in Henoch-Schonlein purpura with nephritis compared to patients without nephritis. Therefore, the concentrations of sOX40L were found to be closely associated with disease activity in the patients. Yan J et al¹⁰ discovered that serum sOX40L levels and the expression of OX40 on CD4⁺ T cells apparently increased in acute coronary syndrome patients, and believed that the upregulated OX40/OX40L signal created an inflammatory environment and

Table 3. Serum Concentrations of sOX40L and Pulmonary Function Data in Patients with Mild, Moderate, and Severe Grade Asthma ($\bar{x} \pm s$)

| Group | No. | FEV1% (%) | FVC% (%) | FEV1/FVC (%) | sOX40L (ng/L) |
|----------|-----|--------------|--------------|--------------|---------------|
| Control | 19 | 97.55 ± 5.88 | 98.98 ± 5.96 | 79.24 ± 4.57 | 3.98 ± 2.83 |
| Mild | 18 | 88.75 ± 8.48 | 98.27 ± 7.50 | 74.36 ± 7.93 | 4.87 ± 1.89 |
| Moderate | 24 | 62.00 ± 8.22 | 70.33 ± 6.13 | 64.38 ± 7.46 | 6.97 ± 5.91 |
| Severe | 16 | 39.07 ± 7.46 | 54.96 ± 7.34 | 47.20 ± 8.46 | 8.71 ± 5.18 |
| F value | | 213.88 | 186.72 | 61.37 | 4.14 |
| P value | | <0.01 | <0.01 | <0.01 | <0.01 |

caused the instability of atheromatous plaque. Therefore, sOX40L could be one of indicators to determine the disease severity. It is suggested that sOX40L might be an important inflammatory mediator in many diseases.

Few relevant results on the role of the inflammatory mediator sOX40L and its functional contribution in bronchial asthma have been reported. Recently, Ezzat MH et al¹¹ found that serum sOX40L levels were significantly higher in asthmatic children than control children. The up-regulation of serum sOX40L correlated with the severity of childhood bronchial asthma. Thus, sOX40L may be a useful biomarker for monitoring inflammation of asthmatic children. Kim MY et al²⁵ reported that there were some differences in OX40L expression on inducer cells between neonatal and adult mice. Are there any differences in the concentrations of sOX40L between children and adult patients? Our study also found that the serum concentrations of sOX40L had apparently increased in adult patients with acute bronchial asthma compared to those in the control group. The serum concentrations of sOX40L in the stable asthmatic patients group clearly decreased to healthy control group levels. Therefore, we believed that sOX40L could be the index for assessing the acute exacerbation of bronchial asthma, that means that the more serious the disease, the higher concentrations of sOX40L. Serum concentrations of sOX40L showed a negative correlation with pulmonary function data, indicating this index might be one of the inflammatory mediators determining the severity of bronchial asthma. However, a larger sample is necessary to validate the importance of sOX40L.

What is the role of sOX40L in asthma? SOX40L may bind to OX40 expressed on CD4+ T cells (data not shown) to persistently activate these T cells, which have been proven to play an important role in the pathogenesis of asthma.²⁶ The higher the concentrations of sOX40L, the more activated T cells there are to exacerbate inflammation. Thus, sOX40L may participate in the development of bronchial asthma by maintaining activation of T cells.

Conclusion

Our study demonstrated that the concentrations of sOX40L escalated higher in adult patients with acute bronchial asthma. Our study also demonstrated the association of sOX40L with disease severity and pulmonary function in asthma. Therefore, sOX40L could be an important inflammatory mediator in asthma. However, further research is needed to determine the precise role of sOX40L in the pathogenesis of asthma.

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