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Expression and significance of TGF-β1 in infant asthma model

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ABSTRACT

This article aimed to study the expression and role of TGF-β1 in young rat asthma. The experimental method in this paper is to replicate the asthma model by aerosolization of egg protein. The young rats with asthma were sacrificed after 2, 4 and 8 weeks. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect the mRNA of TGF-β1 and α-SMA. The expression level was detected by the immunohistochemical staining method to detect the protein expression of TGF-β1, α-SMA, Smad3, Smad4 and Smad7 in bronchopulmonary tissues. At the same time, the image analysis method was used to measure the airway morphological parameters of young rats. The experimental results showed that the expression of TGF-β1 in the lung tissue of young rats in the asthma group was increased after two weeks of the challenge. Compared with the control group, the difference was significant (P<0.05); the expression of TGF-β1, α-SMA and mRNA in the 4-week group was significantly higher, compared with the control group and the 2-week challenge group, the difference was significant (P<0.05). Therefore, the change of asthma airway remodeling is a dynamic process. The TGF-β1 pathway presents a dynamic process in the airway remodeling model of young asthmatic rats. Among them, TGF-β1 induces the division and proliferation of airway fibroblasts and promotes fibroblasts. The cells transform into myofibroblasts and increase the synthesis of fibronectin and collagen, suggesting that it plays an important role in asthma inflammation and pulmonary fibrosis.

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Introduction

Bronchial asthma (asthma) is a chronic inflammatory disease of the airway involving a variety of cells and cellular elements. Airway inflammation and remodeling are the two main pathological features of asthma. Airway remodeling can aggravate airway hyperresponsiveness, leading to persistent and progressive damage to lung function (1). At present, the general guidelines for asthma prevention and treatment at home and abroad emphasize the control of airway inflammation, but not enough attention is paid to airway remodeling after airway inflammation. Therefore, research on the pathological proliferation and hypertrophy of airway smooth muscle cells during airway remodeling and the protection and repair of smooth muscle cells in the early onset of asthma has become a new direction for asthma prevention and treatment and is of great significance to the prognosis of asthma patients, especially early patients. The TGF-β1 signaling pathway is considered to be the main regulatory pathway for airway remodeling in asthma, directly affecting the deposition of airway wall collagen and promoting the formation of fibrosis (2).

The study of Raposo et al (3) concluded that compared with normal subjects, the number of fibrocytes co-expressing CD45, type I collagen and α-SMA in the bronchial mucosa and alveolar lavage fluid of mild asthmatic patients increased, and these cells mostly aggregated nearby The basement membrane area, and the number of submucosal fibrocytes is positively correlated with the thickness of the basement membrane. Sam et al. found that Smads protein is the only known intracellular kinase substrate of the TGF-β1 receptor, and the biological activity of TGF-β1 that leads to airway remodeling is achieved through the Smads protein signal transduction pathway. . Therefore, further elucidating the specific mechanism of TGF-β1 signaling pathway can provide new ideas for the research and prevention of asthma (4).

Thickening the basement membrane and smooth muscle membrane during airway remodeling is an important reason why asthma is not easy to cure. The results of this experiment suggest that the increased expression of TGF-β1 in the airway of asthmatic rats is closely related to the thickening of airway smooth muscle and the thickening of the reticular basement membrane. Inhibition of the production of TGF-β1 or its biological function is effective in preventing the occurrence and development of asthma. Important meaning. The experimental results showed that the expression of TGF-β1 in the lung tissue of young rats in the asthma group was increased after two weeks of the challenge. Compared with the control group, the difference was significant (P<0.05); the expression of TGF-β1, α-SMA and mRNA in the 4-week group was Significantly

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higher, compared with the control group and the 2-week challenge group, the difference was significant (P<0.05).

Materials and Methods

Mice

40 healthy adult male SPF-grade SD rats (weight 150-200g) were purchased from the investigation local experimental animal center.

Asthma airway model

In this study, the sensitization method of young rats was selected according to the method of Palmans et al. and improved. Low-dose OVA was used, and freshly prepared aluminum hydroxide gel and B. pertussis vaccine were used as immune adjuvants and injected via the tail vein. And intraperitoneal injection, two times sensitization, and tail vein injection to maintain sensitization. Experiments have proved that the improved sensitization effect is good. This study proved that the improved sensitization effect is good, using aerosol inhalation of OVA for continuous excitation and tail vein injection to maintain sensitization, and successfully constructed a young rat asthma airway remodeling model. The steps flowchart for establishing an asthma airway model is shown in Figure 1.

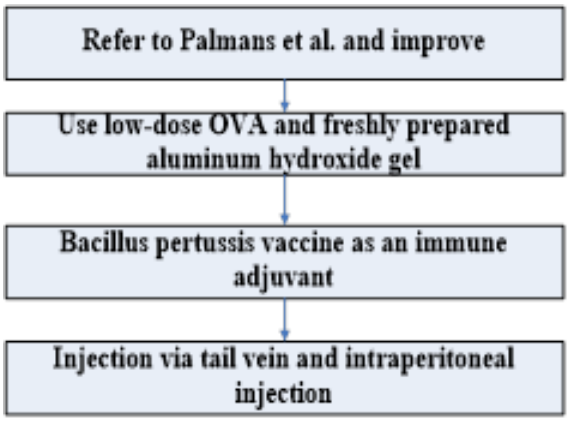

Figure 1. The airway model of asthma should be established step by step.

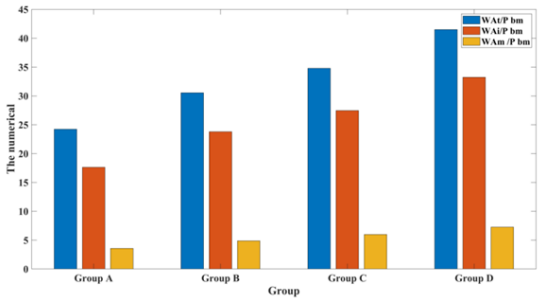

Figure 2. The morphological parameters of the airway were compared between the control group and the asthma group.

Making and Drawing Materials for Rat Asthma Model

Forty SD rats were randomly divided into four groups, with 10 rats in each group. The asthma model was set up according to the references investigated in this article. All asthma groups were sensitized by intraperitoneal injection of OVA (1mg) + Al(OH)3 (100mg) saline mixture on the 1st and 8th day. 1% OVA nebulized excitation, 30 minutes each time, once every other day, the SD rats have shortness of breath, abdominal muscle twitching, and cyanosis of the lips as a representative of successful model replication. Set normal healthy rats as the normal control group (group A), sensitized with 1 mL of normal saline, and inhaled distilled water as a control. The asthma components were asthma 2 weeks group (B group), asthma 4 weeks group (C group) and asthma 8 weeks group (D group). They were killed after aerosol inhalation for 2 weeks, 4 weeks, and 8 weeks, respectively. , Take the lung tissue for HE staining, pathological image analysis, immunohistochemistry and RT-PCR.

Observation of airway subepithelial fibrosis

Place the above-mentioned EVG-stained mouse lung tissue sections under a microscope. Observe at least 10 fields of view under a high-power microscope (x400) for each section to find a complete cross-section of the bronchioles. Use NIS- as the center of the bronchioles. The Elements system measures and analyzes the thickness of the collagen fibers under the bronchioles. Each mouse measures 6 bronchioles at random, at least 3 in each group of mice, and finally calculates the average thickness.

Statistical analysis

All experiments were performed at least three times independently unless otherwise stated. Statistical analysis was performed with SPSS17.0 software, and linear correlation analysis was used for the relationship between the two variables.

Results

Construction of asthmatic rat model

The image analysis results showed that the bronchial tube wall and smooth muscle thickening in the young rats two weeks after the challenge of asthma, and the total tube wall area (WAt/P bm) and inner wall area (WAi/P bm) of the young rats 8 weeks after the challenge And smooth muscle area (WAm /P bm) were significantly increased compared with the control group, 2 weeks and 4 weeks after challenge. The airway smooth muscle gradually thickened with the prolongation of the challenge time. The airway morphology parameters of young rats in the control group and asthma group the specific data are shown in Table 1 and Figure 2. The mRNA expression of TGF-β1 and α-SMA in bronchopulmonary tissues was as

Table 1. The morphological parameters of the airway were compared between the control group and the asthma group.

Group	WA t/P bm	WA i/P bm	WAm /P bm
Group A	24.22 ±1.93	17.62±2.15	3.57 ±0.45
Group B	30.54 ±2.23	23.78 ±2.11	4.87±0.73
Group C	34.78±2.12	27.45 ±2.52	5.97 ±0.79
Group D	41.49±2.65	33.22±2.32	7.25±0.61

the expression of TGF-β1 in the lung tissue of young asthma rats increased after two weeks of the challenge. Compared with the control group, the difference is significant (P< 0.05); the expression of TGF-β1, α-SMA and mRNA in the 4-week group was significantly increased, compared with the control group and the 2-week challenge group, the difference was significant (P<0.05), the 8-week group TGF-β1, α -The expression of SMA and mRNA increased significantly, and the difference was significant compared with the control group, the 2-week challenge group and the 4-week group (P<0.05) The expression of TGF-β1, α-SMA and mRNA in the lung tissues of the control group and the asthma group As shown in Figure 3.

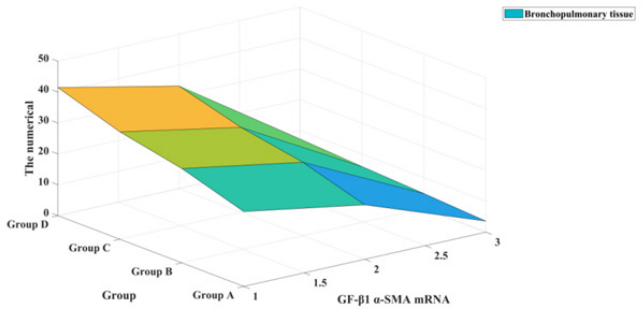

Figure 3. The expression of TGF-β1 and α-SMA mRNA in lung tissues of control group and asthma group.

Detection of the expression of TGF-β1, α-SMA, Smad3, Smad4, and Smad7

The results of the protein expression of TGF-β1, α-SMA, Smad3, Smad4, and Smad7 in bronchopulmonary tissues were as follow. Immunohistochemical staining indicated that the bronchial and lung tissue sections of the 4 groups of young mice showed a brown-yellow immunopositive reaction. The positive expression was mainly concentrated in airway epithelial cells, submucosa, and smooth muscle layer. The gray value determination results are shown in Table 2 and Figure 4. The protein expression of TGF-β1, α-SMA, Smad3, and Smad4 in the lung tissue of the asthma group increased after 2 weeks of the challenge, while the protein expression of Smad7 decreased more significantly, which was compared with the control group. Compared with, the difference is significant (P<0.05).

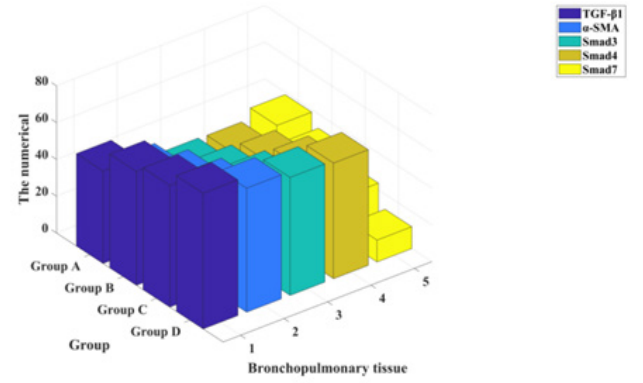

Figure 4. Protein expression of lung tissue indexes in the control group and asthma group.

The protein expression of TGF-β1, α-SMA, Smad3, and Smad4 in the lung tissues of the asthma group increased after 2 weeks of the challenge, while the protein expression of Smad7 decreased. Compared with the control group, the difference was significant (P <0.05). The protein expression of TGF-β1, α-SMA, Smad3, and Smad4 in the 4-week group increased significantly, while the protein expression of Smad7 decreased significantly. Compared with the control group and the 2-week challenge group, P<0.05, the 8-week group TGF The protein expression of -β1, α-SMA, Smad3, and Smad4 increased more significantly, while the protein expression of Smad7 decreased more significantly. Compared with the control group, the 2-week challenge group, and the 4-week group, with P<0.05, the asthma group was larger. The protein expression of lung tissue indexes in the control group and asthma group is shown in Figure 4. The changes in the lung tissue of rats after 2 weeks of challenge are shown in Figure 5.

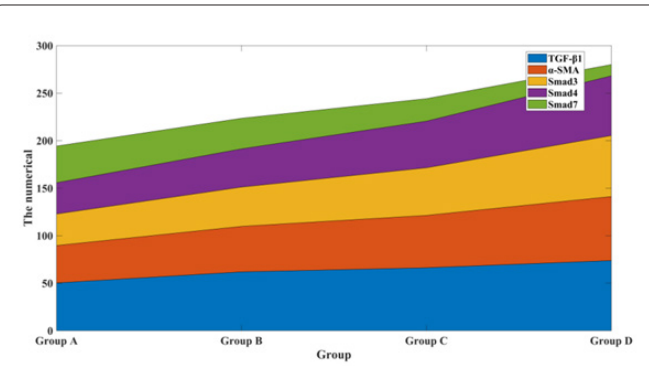

Figure 5. Changes in lung tissue after 2 weeks of stimulation in asthmatic rats.

Discussion

Asthma is a chronic disease. It is a common disease and most often stays on the description of asthma symptoms. Research on the pathogenesis of asthma is still a disease, but its pathogenesis is very complicated. The current understanding of human asthma is very limited, so the prevention and treatment of asthma still stay at the stage of symptomatic treatment, and the cause of treatment is difficult to break through. The research on asthma airway remodeling is difficult to current research (1-3).

The research of asthma airway remodeling has become a bright spot in the research of bronchial asthma in recent years (5). The so-called airway remodeling is one of the

Table 2. Protein expression of lung tissue indexes in the control group and asthma group.

Group	TGF-β1	α-SMA	Smad3	Smad4	Smad7
Group A	50.22±1.67	39.37 ±1.11	33.20±0.88	32.86 ±1.21	38.63 ±0.98
Group B	61.85±2.31	47.88±1.42	41.34 ±1.18	40.23 ±0.94	32.22±1.13
Group C	66.17 ±1.88	55.12± 1.23	50.11 ±0.98	49.03±1.32	23. 73 ±1.42
Group D	73.76 ±2.09	67.38 ±2.11	64.14 ±1.22	62.86 ±1.27	11.88±0.95

pathophysiological characteristics of chronic bronchial asthma (abbreviated as asthma), which is manifested by thickening of airway smooth muscle, thickening of airway basement membrane and deposition of extracellular matrix, inflammatory cell infiltration and glandular hyperplasia Hypertrophy. The result is continuous irreversible airflow obstruction and airway hyperresponsiveness. Because the repaired tissue structure is different from the normal tissue, and the response to external stimuli such as drug treatment is also different, the major challenge for the treatment of bronchial asthma patients is the understanding and treatment of airway remodeling (6). At present, researchers have used different animals to replicate bronchial asthma airway remodeling models, hoping to find an ideal research platform, and provide help for exploring the mechanism of bronchial asthma airway remodeling and corresponding treatment strategies (7). Ovalbumin is usually used to construct an asthmatic airway remodeling model in young SD rats. It provides an ideal model for the study and reversal of the related mechanism of asthma airway remodeling.

After the sensitization and stimulation of young rats, the immediate performance: shortness of breath, irregular rhythm, slow movement, severe incontinence; continuous stimulation performance: slow response, vertical hair, loss of luster. Regarding histopathological changes, in the control group, small bronchi and alveolar structures were normal, the bronchial mucosa epithelium was intact, and no inflammatory cell infiltration was seen. In the asthma model group, the airway wall was thickened, the lumen was narrowed, and the airway mucosal folds increased. Epithelial cells fall off, the airway smooth muscle layer and reticular basement membrane layer thicken, and a large number of eosinophils and lymphocytes infiltrate the submucosa and tube wall. The airway remodeling animal model of asthma had significant changes in airway hyperresponsiveness, epithelial cells, subbasement membrane, and airway smooth muscle (8-9). The pathological features were the most obvious at 6 weeks of modeling, and death occurred in young rats at the seventh week (10-11). There are many kinds of animals for constructing asthma airway remodeling models, among which guinea pigs are the most common (12-14). However, due to the large differences and the difficulty in obtaining the corresponding molecular biological materials, many characteristics of human bronchial asthma can be replicated in rats (15-16). In recent years, the use of rats to make asthma airway remodeling models has increased (17). As a model animal for airway remodeling of asthma, young rats have many advantages, such as wide sources, fast reproduction, easy feeding, and easy availability of biological reagents. The young rat asthma airway remodeling model has many features similar to human childhood asthma, including the allergic reaction is mediated by IgE (18-19), and it is prone to biphasic reactions in the immediate and delayed phases after excitation, and the time of the delayed response. It is closer to the delayed response time of asthma patients (20).

Bronchial asthma is abbreviated as “asthma” (21). Asthma is a serious global health problem. The prevalence of asthma has increased significantly in the past few decades. It is currently estimated that about 300 million people worldwide suffer from asthma (22). Asthma is one of the most common chronic airway inflammatory diseases involving cells and cellular components. A large number of experimental studies have proved that the inflammatory changes, obstruction and remodeling of bronchial asthma can occur in various parts of the entire airway (23). The early manifestations of asthma patients are the most significant with small airway disease. Therefore, monitoring of abnormal small airway function plays an important role in the diagnosis, prevention, and efficacy evaluation of asthma. The pathogenesis of small airway diseases related to asthma has not been fully clarified in reports (24). The results reported in the literature are inconsistent. People think that the central airway is more important than peripheral airway inflammation. On the contrary, some people have different opinions. These inconsistent observations can come from differences in case data, animal models, sampling and testing methods. Therefore, it is necessary to adopt more accurate and reasonable techniques, such as pulmonary function testing and imaging, to clarify the application of small airway disease in asthma.

This experiment found that the change of asthma airway remodeling is a dynamic process. The TGF-β1 pathway presents a dynamic process in the airway remodeling model of young asthmatic rats. Among them, TGF-β1 induces the division and proliferation of airway fibroblasts and promotes fibroblasts. The cells transform into myofibroblasts and increase the synthesis of fibronectin and collagen, suggesting that it plays an important role in asthma inflammation and pulmonary fibrosis.

Acknowledgments
None

Conflict interest
The authors declare no conflict of interest.

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