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Changes in expressions of TIPE2 and TF in peripheral blood mononuclear cells of patients with acute exacerbation of bronchial asthma and their associations with changes in inflammatory factors and T lymphocytes

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Abstract. – OBJECTIVE: To detect the expressions of tumor necrosis factor-α-induced protein-8-like 2 (TIPE2) and tissue factor (TF) in peripheral blood mononuclear cells (PBMCs) of patients with acute exacerbation of bronchial asthma (BA), and to analyze their associations with changes in inflammatory factors and T lymphocytes.

PATIENTS AND METHODS: A total of 59 patients with BA treated in our hospital from February 2018 to April 2019 were selected as objects, including 30 cases in the acute exacerbation phase (BA group) and 29 in the remission phase (RE group). During the same period, 28 people receiving physical examinations were selected as healthy controls (Control group). The proportion of eosinophils in the sputum and the fractional exhaled nitric oxide (FeNO) level were detected in each subject. Blood samples were collected in patients of BA group and Control group, aiming to isolate PBMCs. Then, the messenger RNA (mRNA) expressions of TIPE2 and TF in PBMCs were determined via reverse transcription-polymerase chain reaction (RT-PCR). Serum levels of interleukin-1β (IL-1β) and IL-6 in BA group and Control group were determined via enzyme-linked immunosorbent assay (ELISA), and protein expressions of serum T helper 1 (Th1) and Th2 cells in BA group and Control group were detected using Western blotting.

RESULTS: Compared with those in Control group, the proportion of eosinophils and FeNO level increased in BA and RE group, which were more pronounced in BA group. Downregulated mRNA level of TIPE2, and upregulated TF were detected in BA and RE groups compared to those of Control group, and the expression changes were more significant in the former group. Enzyme-linked immunosorbent assay (ELISA) data showed that serum levels of IL-1β and IL-6 were significantly elevated in BA and RE groups in comparison to Control group, especially BA group. In addition, protein level of Th1 cells was downregulated, while that of Th2 was upregulated in BA group and RE group compared to those of Control group, and a more significant change was observed in BA group compared to that of RE group.

CONCLUSIONS: In patients with acute exacerbation of BA, the expression of TIPE2 in PBMCs declined, while that of TF rose, which were negatively correlated with each other. Moreover, the proportion of Th1 cells declined in patients with acute exacerbation of BA, indicating that it is associated with the lung function, inflammatory level and proportion of eosinophils.

Key Words:
TIPE2, TF, Bronchial asthma, Inflammatory factors, T lymphocytes.

Introduction

Bronchial asthma (BA) is a clinically common chronic respiratory disease, and an allergic disease involving multiple cytokines, such as eosinophils, neutrophils and T lymphocytes. A chronic inflammatory response following BA will lead to the increased airway hyperreactivity, usually manifesting as reversible airflow limitation, recurrent wheezing, chest distress or cough[1,2]. According to an epidemiological survey, the incidence rate of BA has continued to rise with the global industrialization and climate changes,

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and BA not only threatens the health, but also increases the economic burden of patients' families[3]. Therefore, exploring the pathogenesis of BA and searching for therapeutic targets are research hotspots currently.

The expression of tumor necrosis factor-α-induced protein-8-like 2 (TIPE2) declines in the immune system diseases[4,5]. As a novel anti-inflammatory protein discovered in 2008, TIPE2 can regulate and maintain the homeostasis through mediating the body's immune response[6]. Tissue factor (TF), a pro-angiogenesis factor, is involved in airway remodeling in asthma. TF and TIPE2 synergistically promote the occurrence and development of asthma *via* formation and remodeling of bronchial vessels[7,8]. However, the pathogenesis of BA remains unclear, and further exploration is still needed.

With the in-depth research on the pathogenesis of asthma in recent years, researchers have found that the immuno-inflammatory imbalance plays an important role in the incidence of asthma[9]. In healthy people, T helper 0 (Th0) cells differentiate into Th1 and Th2 cells, and they are in a state of dynamic balance that jointly regulate the immune function of normal cells in the body[10]. However, Th2 cell response is dominated in patients with asthma, causing the deterioration of asthma[11]. Therefore, it is believed that the Th1/Th2 imbalance is of great significance for the occurrence and development of BA.

This study aims to detect expression changes of TIPE2 and TF in peripheral blood mononuclear cells (PBMCs) of patients with acute exacerbation of BA, and to analyze the value of their changes in inflammatory factors and T lymphocytes in the pathogenesis of asthma. Our findings may provide new strategies for the diagnosis and treatment of asthma.

Patients and Methods

Reagents

First-strand complementary DNA (cDNA) synthesis kits and fluorescence quantitative assay kits were purchased from Tiangen (Beijing, China); TRIzol solution, and primers of TIPE2, TF and β-actin were purchased from Invitrogen (Carlsbad, CA, USA); primary antibodies against Th1, Th2 and β-actin were obtained from eBioscience (San Diego, CA, USA); Fluorescein isothiocyanate (FITC)-labeled secondary antibodies were bought from Abcam (Cambridge, MA, USA), and enzyme-linked immunosorbent assay (ELISA) kits were obtained from R&D Systems (Minneapolis, MN, USA).

Instruments

A nitric oxide analyzer was purchased from NIOX MINO; a fluorescence quantitative polymerase chain reaction (PCR) was provided by Bio-Rad (Hercules, CA, USA), and a microplate reader was purchased from TECAN (Chapel Hill, NC, USA). An ice machine was bought from Henan AMS Refrigeration Equipment Co., Ltd. (Zhengzhou, China); A -80°C refrigerator was obtained from Thermo Fisher Scientific (Waltham, MA, USA), and a chemiluminescence instrument was bought from Beijing Beier Bioengineering Co., Ltd. (Beijing, China).

Baseline Data

A total of 30 patients with acute exacerbation of BA (BA group) and 29 patients with remission of BA (RE group) treated in our hospital from February 2018 to April 2019 were selected as objects. In RE group, there were 15 males and 14 females aged 29-57 years, with an average of 46.4 years. In BA group, there were 14 males and 16 females aged 25-62 years, with an average of 45.3 years. A total of 28 healthy people undergoing physical examinations during the same period were selected as healthy controls (Control group), including 14 males and 14 females aged 27-67 years, with an average of 49.2 years. BA patients with severe diseases such as hypertension, diabetes, coronary heart disease, autoimmune diseases, tumors and tuberculosis were excluded. There were no significant differences in age and gender among groups (p>0.05). The informed consent was obtained from each subject or their guardians. This investigation was approved by the Ethics Committee of Zhongnan Hospital of Wuhan University. Signed written informed consents were obtained from all participants before the study.

Detection of Lung Function

The lung function was detected in each group using a lung function meter, including the following detection indexes: Forced expiratory volume in one second (FEV_1%), (FEV_1)/forced vital capacity (FVC) and peak expiratory flow (PEF%). Each index was measured for at least 3 times, and the average one was calculated.

Detection of Eosinophil Count in Sputum

According to the references, the sputum was collected after ultrasonic aerosol inhalation of 3%

hypertonic saline for 5 min in each group[12]. Then, the sputum was incubated in 0.1% DTT solution at a ratio of 1:4 at 37°C for 30 min and centrifuged for 10 min. Cells were smeared on the smear and stained. They were classified and counted under a light microscope, and the percentage of eosinophils was calculated.

Detection of Fractional Rxhaled Nitric Oxide (FeNO) Level

The FeNO level was detected using a nitric oxide meter. FeNO is usually measured during single-breath exhalations against a resistance to eliminate the influence of nasal contamination on FeNO. When the exhaled air (50 mL/s) reached a plateau, the FeNO level (ppb) was detected for 3 times (1 ppb = 1×10^{-9}mol/L).

Detection of mRNA Levels of TIPE2 and TF in PBMCs Via qRT-PCR

The peripheral blood was drawn in subjects of BA group and Control group for isolating PBMCs. Cells were lysed in TRIzol to extract the total RNAs. According to the instructions of RT kits, the total RNA was reversely transcribed into cDNA, and then PCR amplification was performed in a 50 μL system for 30 cycles in total. The bands were photographed using a gel imager and the optical density of bands was statistically analyzed using ImageJ software. Real-time PCR was performed with a FastStart Universal SYBR Green Master kit (Roche, Basel, Switzerland). Expression data were normalized by β-actin levels using the 2-ΔΔCT method. The primer sequences used were shown in Table I.

Detection of levels of IL-1Ð and IL-6 in Peripheral Blood Using ELISA

According to the instructions of human IL-1β and IL-6 ELISA kits, the standard curves were drawn first, based on which protein concentrations were analyzed. Then, the absorbance was measured using the microplate reader, and serum levels of IL-1β and IL-6 were statistically analyzed.

Table I. Primers..

List	5′-3′ primer
TIPE2	CGGCACTTAGCTTTGGTGAG
	GAGTGAAGTCAGGCCCATAGA
TF	TGATTGCATCAGGGCCATTG
	GCCAGGTAAGCATCATACACCA
β-actin	CTCCATCCTGGCCTCGCTGT
	GCTGTCACCTTCACCGTTCC

Detection of Protein Levels of Th1 and Th2 in Peripheral Blood Through Western Blotting

The peripheral blood was drawn in subjects of each group, lysed in lysis buffer, and centrifuged. After the precipitate was discarded, the supernatant was taken. Then, the protein was quantified, loaded for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a membrane. Samples were incubated with Th1 and Th2 antibodies (1:1,000) overnight and FITC-labeled secondary antibodies at room temperature for 2 h. Finally, the image was developed using a chemical imager, and the optical density of bands was analyzed using ImageJ software.

Statistical Analysis

Statistical Product and Service Solutions (SPSS) 19.0 software (IBM, Armonk, NY, USA) was used for data analysis. The data were expressed as mean ± standard deviation. The *t*-test was used for the comparison among groups. Pearson correlation analysis was performed for correlation analysis. $p<0.05$ was considered to be statistically significant.

Results

Lung Function Declined in Patients with Acute Exacerbation of BA

Compared with those in Control group, lung function indexes FEV_1, FEV_1/FVC and PEF% declined in BA group and RE group, and the differences were statistically significant ($p<0.05$). Compared with those in RE group, FEV_1, FEV_1/FVC and PEF% significantly declined in BA group ($p<0.05$) (Table II). It is concluded that the lung function declined in patients with acute exacerbation of BA.

Proportion of Eosinophils in Sputum Rose in Patients with Acute Exacerbation of BA

The proportion of eosinophils in the sputum significantly rose in patients of BA group and RE group compared with that in Control group ($p<0.05$), and it also significantly rose in BA group compared with that in RE group ($p<0.05$) (Table III). The above results demonstrated that the number of eosinophils in the sputum increased in patients with acute exacerbation of BA.

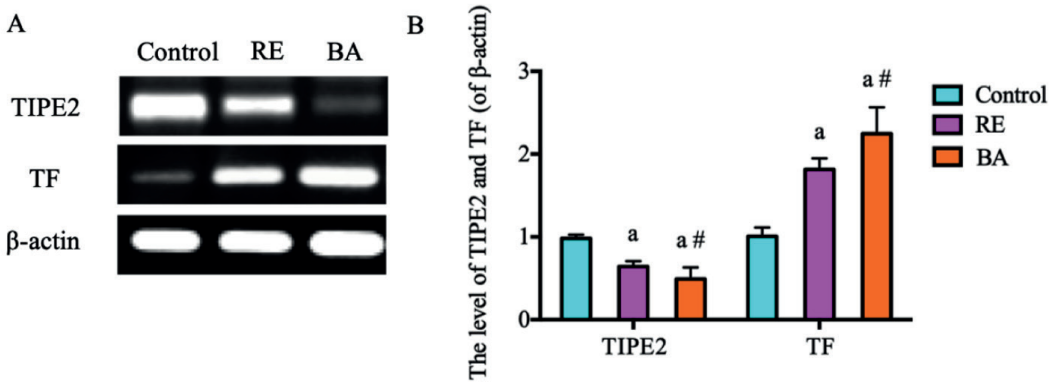

Figure 1. Comparison of TIPE2 and TF mRNA levels using RT-PCR. **A,** RT-PCR bands. **B,** Statistical graph. ([a]: *vs.* Control group, [#]: *vs.* RE group).

Table II. Comparison of lung function.

Group	n	FEV1%	FEV1/FVC	PEF%
Control	28	90±9	92±12	83±6
RE	29	72±6[a]	75±8[a]	75±8[a]
BA	30	61±7[a#]	62±5[a#]	55±4[a#]
t		7.82	5.96	5.71
p		<0.05	<0.05	<0.05

[a]: *vs.* Control group, [#]: *vs.* RE group.

FeNO level Increased in Patients with Acute Exacerbation of BA

The level of FeNO was significantly higher in BA group and RE group than that in Control group (p<0.05), and it was also higher in BA group than that in RE group (p<0.05) (Table IV), suggesting that the level of FeNO greatly increased in patients with acute exacerbation of BA.

Patients with Acute Exacerbation of BA had a Decreased Expression of TIPE2 and an Increased Expression of TF in PBMCs

The results of RT-PCR showed that BA group and RE group had a decreased mRNA expression of TIPE2 and an increased mRNA expression of TF in PBMCs compared with Control group. The above changes were more significant in BA group than those in RE group (p<0.05) (Figure 1).

Levels of IL-1β and IL-6 in Peripheral Blood Rose in Patients with Acute Exacerbation of BA

The results of ELISA revealed that BA group and RE group had significantly increased levels of IL-1β and IL-6 in peripheral blood compared with those of Control group (p<0.05). The above changes were more significant in BA group than those in RE group (p<0.05) (Figure 2).

Table III. Comparison of proportion of eosinophils

List	5′-3′	primer
Control	28	6.16±3.45
RE	29	15±4.73[a]
BA	30	23.48±4.29[a#]
t		5.137
p		<0.05

[a]: *vs.* Control group, [#]: *vs.* RE group.

Table IV. Comparison of FeNO level.

Group	n	FeNO/L
Control	28	19.34±4.19
RE	29	29.41±6.28[a]
BA	30	34.26±7.83[a#]
t		4.274
p		<0.05
		<0.05

[a]: *vs.* Control group, [#]: *vs.* RE group.

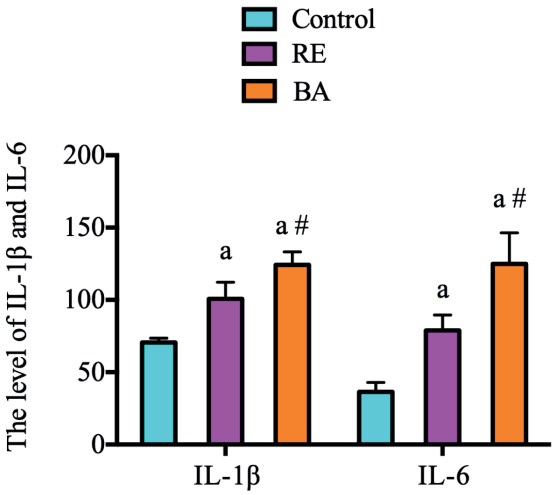

Figure 2. Comparison of IL-1β and IL-6 levels using ELISA. ([a]: *vs.* Control group, [#]: *vs.* RE group).

Patients with Acute Exacerbation of BA had a Reduced Level of Th1 and a Raised Level of Th2 in Peripheral Blood

The results of Western blotting manifested that the protein level of Th1 in PBMCs declined, while that of Th2 rose in BA group and RE group compared with those in Control group ($p<0.05$). The above changes were more significant in BA group than those in RE group ($p<0.05$) (Figure 3).

Correlation Analysis Between Expressions of TIPE2 and TF in PBMCs and Inflammatory Factors in Patients with Acute Exacerbation of BA

According to correlation analysis, the expression of TIPE2 in PBMCs was negatively correlated with those of IL-1β and IL-6 ($r=-0.6834$, $r=-0.6261$, $p<0.05$), while the expression of TF was positively correlated with those of IL-1β and IL-6 ($r=0.5878$, $r=0.7948$, $p<0.05$) (Figure 4A-B). Besides, the expression of TIPE2 in PBMCs was positively correlated with Th1, but negatively correlated with Th2 ($r=0.6719$, $r=-0.6443$, $p<0.05$), while TF had the opposite results ($r=-0.5850$, $r=0.6037$, $p<0.05$) (Figure 4C-D).

Discussion

Based on clinical symptoms, the progression of BA is classified into acute exacerbation phase, chronic persistent phase and remission phase. Patients with acute exacerbation of BA usually suffer from chest distress, shortness of breath, breathing difficulty, vomiting and decline in blood oxygen content, even accompanied by respiratory failure[13]. Despite some progress made in the prevention and treatment of asthma, asthma cannot be cured based on the medical level currently. It not only seriously affects the physical and mental health of patients with BA, but also poses a huge burden on the society. The pathogenesis of asthma is complex, and multiple cytokines are involved in the occurrence and development of asthma. Therefore, exploring the pathogenesis of asthma is an extremely important scientific task.

In this study, patients with BA treated in our hospital and healthy people receiving physical examinations were recruited. It is found that FEV_1, FEV_1/FVC and PEF% markedly declined in BA group and RE group compared with those in Control group, indicating that the lung function of patients with asthma was remarkably reduced. FEV_1 (normal value: 83%) refers to the maximal expiratory volume following the maximal deep breath, and its level will decline prominently in the case of lung obstruction[14]. FEV_1/FVC is the

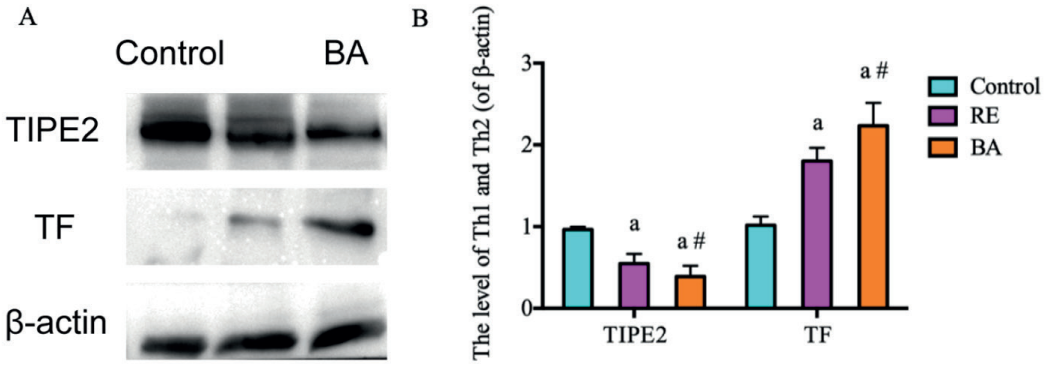

Figure 3. Comparison of Th1 and Th2 protein levels using Western blotting. **A,** Western blotting bands. **B,** Statistical graph. ([a]: *vs.* Control group, [#]: *vs.* RE group)

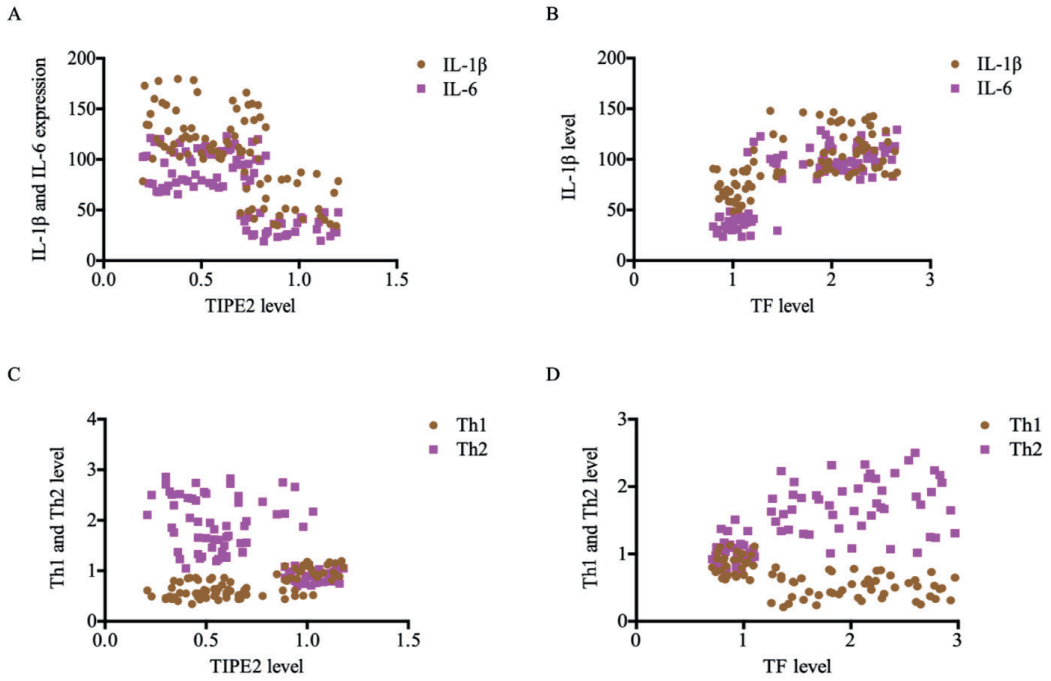

Figure 4. Correlation analysis. **A,** Correlation between TIPE2 and inflammatory factors. **B,** Relation between TF and inflammatory factors. **C,** Correlation between TIPE2 and T lymphocytes. **D,** Association between TF and T lymphocytes

ratio of forced expiratory volume in one second to forced vital capacity, and its level <70% indicates chronic lung obstruction and decline in lung function[15]. PEF% refers to peak expiratory flow, and its level decreases when asthma occurs[16]. Then, the number of eosinophils in the sputum was detected in each group, which remarkably increased in the sputum of patients with BA. Eosinophils are main components of white blood cells, which can release the contents of particles that cause tissue damage and promote inflammation. In the present study, FeNO level markedly rose in patients with BA, indicating the aggravation of inflammatory response since FeNO produced by cells in the airway was highly positively correlated with the number of inflammatory cells. The above results demonstrated that there were massive inflammatory responses in the airway of patients. Furthermore, the levels of TIPE2 and TF in PBMCs were determined *via* RT-PCR. The results manifested that patients with BA had an evidently decreased level of negative immunoregulatory factor TIPE2, but an increased level of TF in the peripheral blood. It is indicated that the levels of TIPE2 and TF in PBMCs of patients with acute exacerbation of BA were negatively correlated, and asthma patients had an imbalance of immune response. According to recent studies, the imbalance of cellular immunity *in vivo* is closely related to the occurrence of asthma. As the main executor of cellular immunity, T lymphocytes are activated by antigens when the body is exposed to allergens for the first time. Then, activated Th2 cells stimulate the production of ILs, such as IL-4, IL-5 and IL-6[17,18], and Th1 cells induces the expressions of TNF-α and IL-1β[19]. These stimulating factors can promote the growth, differentiation and recruitment of eosinophils and mastocytes, thus triggering the occurrence and exaggeration of airway inflammation[20]. Next, the levels of inflammatory factors IL-1β and IL-6 in the peripheral blood were detected. The results showed that the expressions of inflammatory factors were significantly elevated in patients with BA, and these patients had a decreased level of Th1 and an increased level of Th2 in the peripheral blood. Based on the correlation analysis, the expression of TIPE2 in PBMCs was negatively correlated with inflammatory factors and Th2, but positively correlated with Th1, while the expression of TF displayed the opposite results. To sum up, the immune system of patients with BA is destroyed and cannot exert normal immune functions.

Conclusions

To sum up, TIPE2 and TF in PBMCs are involved in the airway inflammatory response in asthma, and they are closely related to the severity of inflammatory response in patients with acute exacerbation of asthma. According to further research, the T lymphocyte type of asthma patients is significantly different from that of healthy people. The novelty of this study was that the detecting expressions of TIPE2 and TF in PBMCs can be utilized to predict the inflammatory response and changes in T lymphocytes in patients with acute exacerbation of BA. Our study provides new ideas for the prevention, diagnosis and treatment of BA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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