

Increased Expression of PI-3K in Asthmatic Rat T Lymphocytes

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Summary: In order to explore the expression of PI-3K in T lymphocytes of asthmatic rats and the relationship between PI-3K and activation of T lymphocytes, 24 Wistar rats were randomly divided into 4 groups: normal control group, asthmatic one-week group, asthmatic two-week group and asthmatic four-week group. T cells were purified from blood of each rat and the expression of PI-3K was observed by immunocytochemical fluorescence staining, the semiquantitative fluorescence intensity was measured by HPIAS-2000 analytic software, and the expression of IL-4 in supernatants was detected by ELISA. The results showed that the fluorescence intensity of T lymphocytes in asthmatic groups was significantly higher than that in normal control ($P < 0.001$), indicating that the expression of PI-3K in T lymphocytes of asthmatic rats was significantly higher than that in those of normal controls, and the difference between acute and chronic stage asthmatic groups was significant ($P < 0.05$). The expression levels of IL-4 protein in supernatants of asthmatic T lymphocytes were significantly higher than those in the normal controls ($P < 0.05$). There was a significant positive correlation between the expression of PI-3K in T lymphocytes and the IL-4 protein expression in supernatants ($r = 0.583$, $P < 0.01$). It was suggested that PI-3K signal pathway may participate in the processes of activation and other cytological effects of asthmatic T lymphocytes, thus may play an important roles in the pathogenesis of asthma.

Key words: PI-3K; asthma; T helper 2 cell; interleukin-4

Allergic asthma is characterized by a variable degree of airflow obstruction, airway hyperresponsiveness, mucus overproduction, and chronic airway inflammation. T helper 2 (Th2) cells are the predominant lymphocyte population that infiltrate the airways of asthmatics, and the cytokine products of Th2 cells, such as IL-4, IL-5, play essential roles in asthma. Many signaling pathways, including PI-3K signaling pathway, participate in the process of differentiation, activation, proliferation and anti-apoptosis of T cells. PI-3K is the key molecule in activating Th2 by CD3 pathway, and it plays an important role in process of proliferation and anti-apoptosis of T cells^[1, 2]. PI-3K is thought to contribute to the pathogenesis of asthma by affecting the recruitment, activation, and apoptosis of inflammatory cells. Furthermore, it has reported that dominant negative form of the class IA PI3K regulatory subunit TAT- $\Delta p85$ applied in asthmatic rat model could notably decrease the Th2 cytokine in BALF, especially the expression of IL-4 and IL-5^[3]. In order to explore the modulation of PI-3K on asthmatic Th2 cells, the expression of PI-3K in asthmatic rat T cells was detected, and its relationship with IL-4 activated and excreted by T cells was studied.

1 MATERIALS AND METHODS

1.1 Materials and Reagents

Twenty-four male Wistar rats, weighing 200—300

g, were obtained from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology. Rabbit anti-PI-3K P110 polyclonal antibody was purchased from Santa Cruz Inc., USA. Rat IL-4 ELISA kit was obtained from eBioscience Inc., USA. Lymphocyte separating medium was purchased from Shanghai Biochemical Reagent Factory (China). OVA and FCS were purchased from Sigma Inc., USA. HPIAS-1000 Type High-resolution Medical Colorful Image Analyzing System (Qianping Image Co., Wuhan). Confocal microscopy (Olympus FV500, Japan)

1.2 Establishment of Asthma Model

Asthmatic rat models were established as previously reported^[4]. Twenty-four rats were randomly divided into 4 groups (each group having 6 rats): normal control group, asthmatic one-week group, asthmatic two-week group and asthmatic four-week group. Each rat in normal control group was intraperitoneally injected with 1 mL PBS, and 2 weeks later, inhaled fog PBS for 30 min per day for 15 days. The rats in 3 asthmatic groups were subcutaneously sensitized with injection OVA suspension (1 mL) in medial thigh (OVA 10 mg+aluminium hydroxide 100 mg), intraperitoneally injected with inactivated chincough bacilli (6×10^9) and repeated after 8 days, and on the 15th day, stimulated with 2% OVA suspension (40 mL) per day for 30 min, till the symptoms of asthma occurred, indicating the successful establishment of asthma.

After challenged, rats presented asthmatic symptoms such as agitation, cough, tachypnoea, cyanosis and so on. The pathology of asthma was characterized by infiltration of eosinophils and lymphocytes in the air-

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ways and constriction of small airway and vessel in the lung slices.

1.3 T Lymphocyte Isolation and Culture

In asepsis operation, blood (6 mL) was taken from each rat through hepocelom venipuncture, diluted, added into lymphocyte separating medium with same volume, then centrifuged at 2200 r/min for 20 min. The layer of milky liquor was aspirated from the centrifuge tube. Ammonium chloride (0.85%) was applied to break up red cells, then cells were washed 2 times with PBS and centrifuged at 1600 r/min for 15 min. RPMI1640 (1 mL) culture solution containing 10% fetal bovine serum (FBS) was added to centrifuge tube, incubated for 1 h at 37 °C at an atmosphere of 5% CO₂ in an incubator. Cells were cultured in asepsis nylon cotton column for 1 h at 37 °C. Collected T cells were adjusted to a density of 2×10⁶/mL in 24-well plates and were fixed with RPMI1640 culture solution containing 10% FBS, and inoculated in an incubator of 5% CO₂ at 37 °C.

1.4 PI-3K Immunofluorescence Staining

Immunofluorescence staining was carried out according to the standard procedures. T cells (1×10⁷/mL, 20 μL) were cultured for 1 h, then added to the slides pretreated with poly-L-diaminocaproic acid for 10 min at room temperature and fixed in crymo-acetone blocking solution for 10 min. The slides were washed 2 times with PBS. After being washed, the cells were blocked with normal goat serum for 30 min in a wet box. The primary antibody against PI-3KP110 Pab was diluted to 1:100. Cells were incubated with the primary antibody at 4 °C overnight. The same process without the primary antibody was used as a control. After incubated with secondary antibody labeled with fluorescein at 37 °C for 1 h, cells were observed under fluorescence microscopy at once and recorded photographically. Fluorescence intensity as the amount of the expression of protein was measured by HPIAS-2000 analytic software.

1.5 IL-4 ELISA Assay

T cell suspension, cultured for 24 h, was centrifuged at 4 °C, 2000 r/min for 15 min, and supernatant was stored at -70 °C. IL-4 protein in the supernatant was measured by using an ELISA kit.

1.6 Statistical Analysis

The data were analyzed by the SPSS software (version 11.5) to assess the statistical difference and expressed as $\bar{x} \pm s$. Differences between groups were analyzed by using one-way analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

2 RESULTS

2.1 Pathomorphological Image Analysis of Asthmatic Rat Models

In asthmatic group rats, the caliciform cells in bronchiole were increased; a large number of eosinophils infiltrated on the peripheral airway and in airway lumen; a large number of lymphocytes infiltrated around the bronchial; and the constriction of small airway and vessel were also observed.

2.2 Expression of PI-3K in T Lymphocytes of Asthmatic Rats

The expression of PI-3K in T lymphocytes of

asthma was significantly higher than that in normal controls, and the expression of PI-3K was also increased in a time-dependent manner. There was significant difference between control group and asthmatic groups ($P < 0.001$). And there was significant difference between asthmatic two-week group and one-week group ($P < 0.05$). But there was no significant difference between asthmatic two-week group and four-week group ($P > 0.05$). The results were shown in fig. 1.

2.3 IL-4 Protein Expression in Supernatant of T Lymphocytes

The expression of IL-4 protein in T lymphocyte supernatant of asthma was significantly higher than that in normal control groups and there was significant difference between two groups ($P < 0.05$). But there was no significant difference in the expression of IL-4 protein among asthmatic groups ($P > 0.05$).

Table 1 Fluorescence intensity of PI3K in T lymphocytes and the levels of IL-4 in cultured supernatants ($\bar{x} \pm s$)

Groups	<i>n</i>	Fluorescence intensity of PI3K	Levels of IL-4 in cultured supernatants (ng/L)
Control (A)	6	7.3562±2.1480	4.0±1.5
Asthmatic one-week (B)	6	10.2094±2.4182*	7.7±2.8 [△]
Asthmatic two-week (C)	6	11.3488±2.6327* ^{△△}	9.0±4.0 [△]
Asthmatic four-week (D)	6	12.1513±2.2048* ^{△△}	9.1±3.6 [△]

* $P < 0.001$, [△] $P < 0.05$ as compared with control group;

^{△△} $P < 0.05$ as compared with asthmatic one-week group

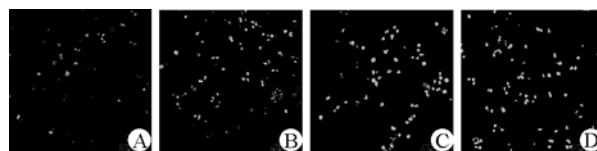


Fig. 1 Immunocytochemical fluorescence staining of PI3K in T lymphocytes (×20)

A: Normal control group; B: Asthmatic one-week group; C: Asthmatic two-week group; D: Asthmatic four-week group (×20). The expression of PI3K in normal control group was weaker than any other groups. The expression of PI3K in three asthmatic groups was significantly increased as compared with normal control group ($P < 0.001$)

2.4 Correlation Analysis

There was a good positive correlation between the expression of PI-3K in T lymphocytes and the levels of IL-4 protein in supernatants in asthmatic rats by linear correlation analysis ($r = 0.583$, $P < 0.01$).

3 DISCUSSION

Asthma is a chronic inflammatory disease characterized by an over activation of CD4⁺ T cells in the peripheral blood and airway tissues. IL-4, IL-5 and other cytokines secreted by Th2 cells play the central role in asthma inflammation course. Usually IL-4 is mainly secreted by Th2 cells, so the expression level of IL-4 protein is customarily considered the activation of Th2 cells.

The increase of the expression of IL-4 protein is due mainly to an increased effect of Th2 cells. Multiple cell signal transduction pathways participate to promote differentiation, activation, proliferation and anti-apoptosis in Th2 cells. Our previous studies have shown that PKC/NF- κ B signal transduction pathway participates in regulating the activation of T cells and the expression of IL-4 protein, thereby it may take part in the course of the asthma inflammation^[5]. Similarly it was reported that MAPK signal transduction pathway played a critical role in regulating the proliferation, activation and cytokine production of the T cells^[6].

In these studies, it was found that other signal transduction pathways might also take part in the T cells effect. Additionally, it has been reported that PI-3K signaling pathway, one of the most important signal transduction pathways, was closely involved in the proliferation, differentiation and activation of T cells^[7-9]. Activated PI3K can generate phospholipid second messenger molecules phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate, which raise a diverse set of cellular responses. The major targets of phosphatidylinositol 3,4,5-trisphosphate and phosphatidylinositol 3,4-bisphosphate are PH domain-containing proteins, such as Akt. Akt can mediate many PI3K-regulated biological responses including glucose uptake, protein synthesis, cell cycle progression, and apoptosis inhibition. Activated kinases including Akt and MAPK1/2 mediate survival signal transduction and cell cycle progression by phosphorylating downstream key regulatory proteins. Although MAPK and PI3K/Akt pathways have been thought to organize distinct cascades, recent reports suggested that a certain degree of cross-talk exists between these pathways^[10]. As a kind of essential molecule in anti-apoptosis signaling pathway, Akt/PKB can catalyze BAD phosphorylation and promote decollement with bcl-2. Activated PI3K can block the binding of Caspase-8 and death induced signal compound (DISC) through CD3 pathway, thus Th2 cells can be protected from Fas-induced death^[2, 11].

In our experiment, it was shown that the expression of PI3K in T cells of asthmatic rats was notably up-regulated ($P < 0.001$), especially in chronic phase cells. At the same time the expression of IL-4 in supernatants was detected by ELISA. The results showed that the expression of IL-4 in asthmatic groups was significantly increased ($P < 0.05$), but there was no significant difference among the three asthmatic groups. There was a good positive correlation between the expression of PI-3K in T lymphocytes and the levels of IL-4 protein in supernatants ($r = 0.583$, $P < 0.01$). This suggested that the activated and increased expression of PI-3K might lead to the activation of asthmatic T cells and play the pivotal role in physiology and pathology course of asthma. Other cytological effects of asthmatic T lymphocytes induced by PI3K, especially cellular proliferation, activation and anti-apoptosis, might due to the difference between acute stage and chronic phase, and the persistence and chronic course of asthma. Therefore, to a certain degree, it was suggested that PI-3K signaling pathway was necessary in

the processes of activation of asthmatic T cells.

To sum up, it was found that PI3K signaling pathway was involved in the activation of asthmatic T lymphocytes and the expression of IL-4 protein, thus it may play an important role in asthmatic inflammation. But whether this function is necessary for asthma is not well known. It is seldom reported that PI-3K signaling pathway participated in the processes of other cytological effects of asthmatic T lymphocytes. So the further studies would be required to explore these detailed mechanisms.

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