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A New Regulation Pathway in Asthma: NF-kB Activates the Expression of IL-17

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ABSTRACT Objective: To investigate the role of NF-kB and IL-17 on the etiology of asthma and to explore the potential relationships between them. **Methods:** Bronchoalveolar lavage fluid (BALF) was obtained by fiberoptic bronchoscope setting into the trachea of asthmatic patients (n=40), healthy individuals (n=20) and non-asthma patients(n=20). The expressions of NF-kB (P65) mRNA and IL-17 mRNA were qualified through the RT-PCR and Western blotting. **Results:** The distribution of expression of P65 mRNA was significantly different between group1-asthma group (acute attack), group 2-non-asthma group and healthy controls. The relative capacity of P65 and IL-17 in asthma group was obviously higher than those of other groups. **Conclusions:** It is indicated that the IL-17 and NF-κB might be involved in the etiology of asthma, especially for the acute one. Our study also suggested a potential cause-effect association between IL-17 and NF-kB, which is the first investigation of the relationship between the two factors.

Key words: Bronchial asthma; Children; NF- κB; Pathogenesis

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Introduction

Asthma is a chronic airway inflammatory disorder which induces bronchial hyperresponsiveness (BHR) and airway obstruction [1]. The morbidity and mortality of asthma is acting an increasing tendency in Chinese children, according to an investigation among Chinese children. Multiple studies have proved increased expression of several inflammatory proteins might play an important role on the pathogenesis of asthma[2-5]. Though the pathways in which inflammatory protein act on asthma are not clear yet, there is a common view that transcription of inflammatory gene is related to transcription factor Nuclear factor-kappa B (NF-kB). Nuclear-translocated NF- kB combines with enhancer or promoter regions of the gene with high-affinity binding motifs for NF-kB to initiate the transcription of the genes through synergy with other transcription factors [8]. Some evidences indicated the genes of inflammatory proteins regulated by NF-kB take part in the pathogenesis of asthma[9-15]. Meanwhile some cytokines which were regulated by NF- kB had been proved to modify the expression of NF-kB [16,17]. Therefore, Lorraine et al proposed that a feed-forward amplifying circle might construct the basis for the persistence of the chronic inflammatory process in asthma[18].

IL-17 playes a vital role on the recruitment of neutrophils and widely exists in bronchial biopsies, bronchoalveolar lavage fluid and sputum of patients with asthma [19-21]. It has also been found over-expression in lung epithelium stimulates chemokine production and worsen leukocyte infiltration in vivo[22]. Sophie Molet et al found that expression of IL-17 was significantly increased in peripheral blood eosinophils and IL-17 enhanced the production of profibrotic cytokines though investigating the expression of IL-17 in sputum and bronchoalveolar lavage specimens with Western blot and immunocytochemistry technology [19]. Dominique MA Bullens et al also found IL-17A mRNA levels were significantly elevated in asthma patients[23]. Some related investigation indicated that IL-17 responses and the levels in the airways and serum correlate with disease severity in asthmatic patients.

In this study we intend to investigate the role of NF-kB and IL-17 in the etiology of asthma by testing the expression of NF-kB (P65) mRNA and IL-17mRNA in Bronchoalveolar lavage fluid (BALF) in asthma children and explore the relationship between NF-kB and IL-17 in the pathogenesis of asthma.

1 Materials and subjects

1.1 Subjects

BALF samples were collected whenever the fiberoptic bronchoscope (BF10; Olympus Corp., Tokyo, Japan) was performed by a professional doctor on 80 individuals (48 males and 32 female) with the average age of 4.5 years in the Affiliated Hospital of Medical College, Qingdao University from December 2012 to February 2013.

The sample consisted of three groups as follows: group1-asthma group (acute attack); group 2-non-asthma group and healthy controls. The asthma group consisted of 24 males and 16 females with the age range from 1.5 years to 10 years and average age of 4.5 years; non-asthma group (pneumonia) consisted of 12 males and 8 females with the age range from 1.5 years to 10 years and average age of 4.5 years. Asthma patients in group 1 were diagnosed by two professional doctors according to Routine children asthma prevention made in 1988 by Pediatric asthma branches in

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China. And group2 and group 3 were matched by age and gender with group 1.

1.2 Materials

BALF were collected by fiberoptic bronchoscope into the trachea, after filtered and centrifuged, sediment of which were collected to do the test following.

1.3 RT-PCR

Quantitation of mRNA Expression by Real-Time Reverse Transcription- Polymerase Chain Reaction Real-time-PCR (RT-PCR) was used to measure expression of P65 and CXCR4 mRNAs. Total RNA was isolated from the BALF using Trizol (Invitrogen Co, Carlsbad, CA, USA). PCR reactions (50 μL) contained 5 μL 10× Taq Buffer, 4 μL 2.5 mM dNTP, 4 μL 25 mM $MgCl_2$, 2 μL each of forward and reverse primers, 0.5 μL Taq polymerase and 2 μL cDNA template. Reactions were amplified for 35 cycles of 45℃ for 15 min, 97℃ for 5 min, and 5℃ for 5 min using a GeneAmp 9700 PCR system Applied Biosystems, Foster City, CA, USA. The following primers were used: P65 (forward), 5'-tcaatggctacacaggacca-3', P65 (reverse), 5'-cactgtcacctggaagcaga-3'; IL-17 (forward), 5'-tggctgaaaaggtggtctatg-3',IL-17 (reverse), 5'-cgatgctgatcccaatgtagt-3'; As an internal control, GAPGH mRNA levels were quantified using the following primers: GAPGH(forward), 5'-tcatgggtgtgaaccatgagaa-3', IL-17(reverse), 5'-ggcatggactgtggtcatgag-3'.

1.4 Western blotting

BLAF were centrifuged at 14000 rpm for 15 min at 4 ℃. Sediment were collected and then homogenized in RIPA Lysis Buffer. Total protein was quantified using the bicinchoninic acid assay kit (Pierce, Rockford, IL, USA). Equal amounts of protein (125 μg) were resolved on 10% Tris-glycine SDS (sodium dodecyl sulphatase) polyacrylamide gels. Protein bands were blotted onto nitrocellulose membranes. After blocking in 5% dried milk in Tris-buffered saline containing Tween-20 for 1 h at room temperature, membranes were incubated for 12 h at 4 ℃ with the following antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA): anti-P65(1:1000), anti-IL-17 (1:2000). Membranes were incubated for 1 h at room temperature with HRP-marked secondary antibody (1:6000). Peroxidase labeling was detected using the enhanced chemiluminescence Western blotting detection system (Amersham Pharmacia Biotech, Piscataway, NJ, USA) and analyzed by densitometry.

1.5 Statistical Analysis

Statistical analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Results are reported as mean ± SD. Differences were analyzed for significance by one-way or two-way analysis of t-test and $P< 0.05$ was considered to be statistically significant.

2 Results

2.1 RT-PCR result

The result of RT-PCR indicated that P65 existed widely in three groups with relative capacity of 0.5211± 0.2233; 0.4002± 0.1822 and 0.2553± 0.0904 respectively. The distribution of expression of P65 mRNA was significantly different between group1, group 2 and controls. The relative capacity of P65 in asthma group was obviously higher than those of controls as well as group 3. Both of the differences were statistically significant. The same distinction tendency was observed in the expression of IL-17. The relative capacity of IL-17 were 0.3128 ± 0.0176; 0.3045 ± 0.1910 and 0.1829 ± 0.0091. The differences among groups were statistically significant. The result of our study indicated that P65 and IL-17 were up-regulated in the asthma acute attack(Fig.1).

Fig .1 The expression levels of P65 and IL-17mRNA by RT-PCR. M: Marker Group 1: asthma group: Group 2: non-asthma group: Group 2: healthy controls.

2.2 Western blotting result

Expression of P65 and IL-17 in BLAF were tested by Western blotting. The result suggested significant differences existed between group1, group 2 and group 3 (P65:0.7026 ± 0.0375; 0.6340 ± 0.0379; 0.1007 ± 0.0793; IL-17:0.4472 ± 0.0193; 0.3091± 0.0216;0.1025± 0.0384). The differences were all stically significant.($P<0.05$, Fig.2). The result demonstrates the conclusion made through result of RT-PCR.

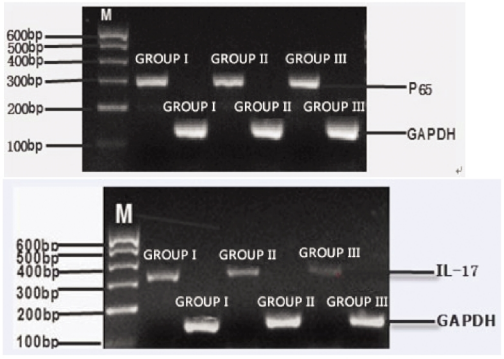

Fig .2 The expression level of the P65 and IL-17 protein. Group 1:asthma group: Group 2:non-asthma group: Group 3:healthy controls

3 Discussion

In this study we have investigated IL-17 and NF-kB expression in the pathogenesis of asthma through comparison between asthma acute attack, non-asthma and healthy controls. We found higher expression levels of both IL-17 and NF-kB mRNA in asth-

ma attack and non-asthma compared with controls, directly demonstrating the involvement of IL-17 and NF-kB in asthma. Besides that levels of NF-kB were highest in asthma attack correlated with the IL-17 mRNA expression, suggesting potential cause-effect association between IL-17 and NF-kB, which is the first investigation of the relationship between the two factors. Moreover, all healthy individuals had low expression of IL-17 and NF-kB which makes it advisable to set IL-17 and NF-kB level as an diagnose standard in asthma.

Although asthma has been long studied and quantities of evidences indicated immunological and inflammatory factors played important roles in the pathogenesis of asthma [24-27], detail mechanisms and related factors were still unknown. Asthma is a T cell mediated chronic inflammatory disorder of the airway[28]. Park H et al[22] and Harrington LE et al[29] found a T cell lineage called Th17 cells or inflammatory T cells producing IL-17, which has proven to be over-expressed in lung epithelium especially in acute, severe exacerbations of asthma in murine asthma models. IL-17 was also been found high-expressed in eosinophils in asthma, which strongly suggested IL-17 might influence the release of proinflammatory mediators to regulate inflammatory responses [19]. IL-17 as also increased level in activated $CD4^+$ T cells [30], and its receptors were detected more widely in almost ubiquitous cellular distribution.[31]. However, some investigations offered an opposite view. Fossiez F et al found IL-17 might not be involved in the secretion of cytokines by T cell[30]. Our results supported the point that IL-17 mRNA and IL-17 protein played an important role in the development of asthma, and the expression quantity might be related with the severity of the disease.

NF-kB was another important factor in asthma and has been found to be involved in transcription of a series of gene products related to lung disease [32]. A quantity of inflammatory protein, especially cytokines and enzymes, were found to be regulated by NF-kB, such as TNF-a interleukin-1b (IL-1b), IL-4, and IL-5; the chemotactic cytokines (chemokines) regulated on activation, normal T-cell expressed and secreted (RANTES), and so on. High relavance between NF-kB and asthma was observed in our experiment as well. Expression level of NF-kB protein was obviously increasing in asthma groups, and a significant statisticdistinction was observed between different groups, especially between asthma group and healthy controls, which strongly indicated NF-kB activation get involved in the pathogenesis of asthma. Similar quantity-severity relative was observed on NF-kB investigation.

Though many research have suggested NF-kB and IL-17 play important roles on the etiology of asthma, seldom of them have foucas on the potential mechanism on them in development. As mentioned above NF-kB used to influence inflammation by regulating the transcription of our related factors invlved in asthma, no investigation try to explore the relationship between the both. Our investigation was the first time to explor the relationship between NF-kB and IL-17 in the mRNA level and protein level. The result indicated NF-kB mighapy of asthma at the same time. Some experts ht modify the progress of athma by regulating IL-17 expression.,which added the mechanism of asthma on some respect and supply effective methods to therave tried to remit asthma by inhibiting the synthesis and expression of NF-kB, however, side-effect was too serious that seldom of them was allowed to clinical trails, which might be due to low specificity of NF-kB regulation pathway. Our research indicated we might treat with asthma by inbiting downstream related factors of the regulation pathway to avoid relecant side effects.

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NF-κB 在哮喘病患儿支气管肺泡灌洗液中的表达及意义

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摘要 目的：探讨 NF-κB、IL-17 在儿童支气管哮喘中的作用及机制。本文通过观察哮喘急性发作儿童支气管肺泡灌洗液(BALF) NF-κB、IL-17 的水平变化,探讨其在哮喘急性发作儿童气道炎症中的作用。**方法：**我院 2012 年 12 月 -2013 年 2 月期间行纤维支气管镜检查患儿共 80 例,包括哮喘急性发作组(哮喘组,n=40)、非喘息组(肺炎组,n=20)及对照组(n=20),收集所有病例的 BALF,进行细胞学分类,RT-PCR 法测定 BALF 中细胞中 NF-κB 蛋白(P65mRNA)、IL-17mRNA 的表达;Western 法检测 P65 蛋白、IL-17 蛋白表达。**结果：**与对照组比较,哮喘组和肺炎组患儿的 P65mRNA、IL-17mRNA 水平均明显增高;P65 蛋白、IL-17 蛋白水平均明显增高(均 $P<0.05$);哮喘组患儿的P65mRNA、IL-17mRNA 水平、P65 蛋白、IL-17 蛋白水平较肺炎组高($P<0.05$)。**结论：**NF-κB、IL-17 在哮喘儿童气道炎症中发挥重要作用,NF-κB 通过调控 IL-17 来实现促进哮喘病情进展。

关键词：支气管哮喘;儿童;NF-κB;发病机理
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