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The correlation between children's status asthmatics and interstitial lung disease

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Abstract. – OBJECTIVE: Investigating the correlation between children's status asthmatics and interstitial lung disease (ILD).

PATIENTS AND METHODS: We continuously selected 20 cases of children's status asthmatics combined with ILD (group A), 20 cases of pure status asthmatics (group B), 20 cases of pure ILD (group C) and 20 cases of healthy children (group D). We measured Th1/Th2 by flow cytometry as well as the level of expression of hs-CRP, IL-7 cytokines (TSLP), monocyte chemoattractant protein-1 (MCP-1) and anti-Jo-1 antibody by ELISA method.

RESULTS: Th1 and Th1/Th2 of groups A and B were significantly lower than those of group C and D. Th2 was significantly ($p<0.05$) higher than that of groups C and D. The level of expression of hs-CRP, TSLP, MCP-1 and anti-Jo-1 antibody in the groups A and B were all significantly higher ($p<0.05$) than those of groups C and D. There were differences of the above index of the comparison between groups A and B, but no difference between groups C and D.

CONCLUSIONS: Children's status asthmatics and ILD may correlate with the abnormal expression of Th1/Th2, hs-CRP, TSLP, MCP-1 and anti-Jo-1 antibody.

Key Words:
Children's status asthmatics, Interstitial lung disease, Th1/Th2, hs-CRP, Sample IL-7 cytokines, Monocyte chemoattractant protein-1, Anti-Jo-1 antibody.

Introduction

Children's status asthmatics was an airway inflammation caused by the imbalance of Th1/Th2, Th2 being the dominant position. Indeed, sample IL-7 cytokines (TSLP) had played an important role through up-regulation of the cell expression of Th2 in the occurrence and development of asthma to make the balance between Th1/Th2 and to make that tilt to Th2[1,2]. Status asthmatics is a severe form of asthma in the result of the combined action of the external environment (dust or house dust mite) and autoimmune disorders[3]. According to the statistics, the occurrence rate of children's status chemoattractant complicated with interstitial disease (ILD) gradually increases. There are about 2 to 3.5 million cases of newly diagnosed children asthma in our country every year, in which 3-10% can be complicated with ILD[4]. ILD is a chronic lung disease characterized by diffusive infiltration and gas exchange impairment with complex pathogenesis, but most scholars believe that autoimmune disorders play an important role in the occurrence of the lung injury[5]. Currently, based on the rare relative study about children's status asthmatics, the study explains the possible mechanism of action by means of analyzing the expression level of relative immune active cells or factors.

Patients and Methods

Patients

The study has obtained the approval of the Ethics Committee of our hospital and informed consent of the relatives of the patients. Continuously selected from January, 2014 to January, 2016, 160 patients including 20 cases of children's status asthmatics combined with ILD (group A), 20 cases of pure status asthmatics (group B), 20 cases of pure ILD (group C) and 20 cases of healthy children (group D) were admitted in our hospital. Diagnosis of children's status asthmatics was in accordance with the standard of the Global Initiative for Asthma (GINA), and the diagnosis of ILD was in accordance with the American Thoracic Society (ATS) of 2002, and the clinical, pathological and radiological criteria of European Respiratory Society (ERS). Inclusion criteria: 1. Age from 5 to 18 years old; 2. The children's status asthmatics can be corrected, idiopathic interstitial lung disease (ILP). Exclusion criteria: 1. Congenital abnormalities, hereditary metabolic diseases; 2. Other types of ILD, such as ILD with obvious causes; au-

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toimmune diseases combined with other types, such as systematic lupus erythematosus, etc. There were 12 males and 8 females in group A, aging from 6 to 17 years old with an average age of (12.3±4.5) years old. 11 males and 6 females were in group B, aging from 5 to 16.5 years old with an average age of (12.0±4.3) years old. 10 males and 10 females were in group C, aging from 6.5 to 17.5 years old with an average age of (12.6±4.4) years old. 11 males and 9 females were in group D, aging from 6 to 17 years old with an average age of (12.8±4.9) years old. Gender and age of the four groups were compared, and the difference was not statistically significant.

Methods

Th1/Th2 was detected by flow cytometry, the expression level of hs-CRP, sample IL-7 cytokines (TSLP), monocyte chemoattractant protein-1 (MCP-1) and anti-Jo-1 antibody were detected by ELISA method.

Flow Cytometry

Main reagents and instruments: lymphocyte separation medium (Tianjing Hao Yang Biologics, Ltd.), BFA, ionomycin, PMA (Alexis Biochemicals, San Diego, CA, USA) Anti-Human IL-4 PE, Anti-Human y-IFN PE, Anti-Human CD4 FITC (eBioscience, San Diego, CA, USA). CO_2 thermostatic cell incubator (Guangzhou South Biomedical Instrument Co., Ltd.), refrigerator of -20°C (Siemens, Munich, Germany), flow cytometry (Beckman Coulter Co., Ltd., Fullerton, CA, USA), optical microscope (Leica), ultra clean bench (Suzhou purification equipment), pipettes (Eppendorf, Hamburg, Germany), centrifuge (Heraeus Company, Hanau, Germany), EDTA anticoagulation tube (Kehua Biology, Shangai, China), cell culture plates (Heraeus Company, Hanau, Germany), EU tube (Hyclone Co., Logan, VT, USA).

1) Specimen collection: fasting collection of venous blood of 5 ml, EDTA anticoagulation, 2000 r/min centrifugal for 10 min within 2h, should be carried out in the early morning, and the cytokines were stored in the supernatant of -20°C for inspection. The remaining blood was used for the density gradient centrifugation (2000 r/min centrifugal for 20 min) to isolate peripheral blood mononuclear cells (PBMC), and the detection of Th1/Th2 was carried out by flow cytometry inside 8h in the machine.
2) The isolation of PBMC: lymphocyte separation liquid (Ficoll, Milton Keynes, UK) was adopted to stratify. The lower layer was red blood cells and neutrophils, the upper layer was plasma and platelets, and the white cloud in the middle layer was PBMC.
3) PBMC plate and activation: we adjusted the concentration of 2×10^6/ml under a microscope,

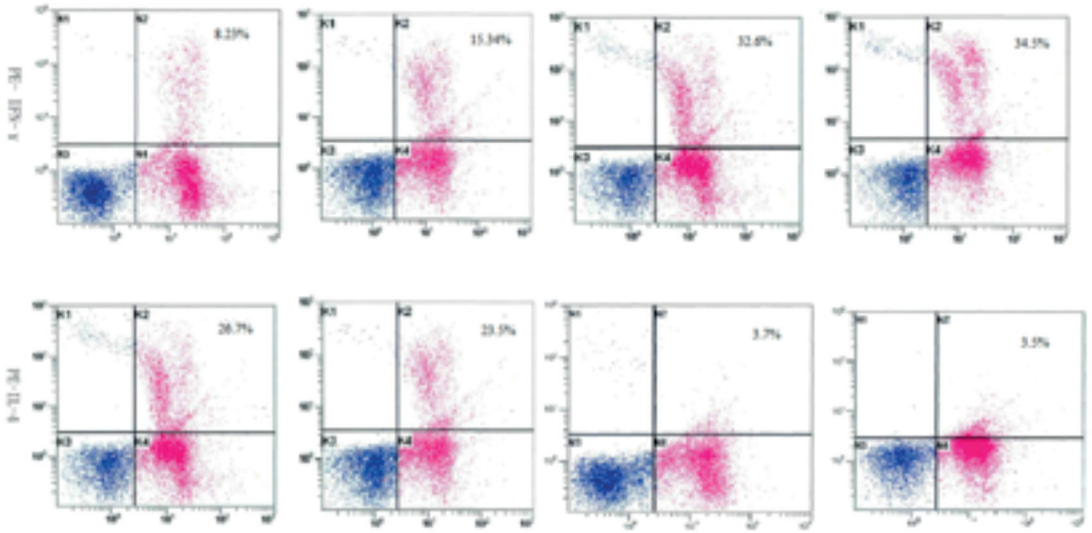

Figure 1. Flow cytometry analysis. Flow cytometry was used to detect Th1, Th2 and Th1/Th2(the upper row is the count of Th1, and the lower row is the count of Th2, and from left to right, they are group A, B, C, and D in sequence.). Group A represents children's status asthmaticus combined with ILD; group B represents the pure status asthmaticus; group C represents the pure ILD; group D represents the healthy children.

Table I. Th1 and Th1/Th2 were detected by flow cytometry (%).

Group	Th1	Th2	Th1/Th2
Group A	7.56±1.32	30.62±5.32	27.53±3.62
Group B	13.03±3.62	27.54±5.16	46.39±8.25
Group C	34.71±4.57	2.63±1.20	1700±200
Group D	36.59±4.10	3.21±1.33	1800±400
F	17.526	14.532	1236.524
p	<0.001	<0.001	<0.001

followed by adding freshly prepared phorbol ester (PMA, 50 ng/ml), ionomycin (2 μg/ml) and Brefeldin streptozotocin (BFA, 3 μg/ml), successively. The suspension was put into the cell culture plate of 24 holes and incubated for 5h in the 37°C 5% CO_2 incubator.

4) Intracellular and cell surface antibody labeling of Th1/Th2: two flow tubes of 1.5 ml were re-suspended, and 1 ml of phosphate buffered saline (PBS) was added. Tubes were further centrifuged (1500 r/min for 5 min) and the supernatant was discarded. The anti-human CD4-FITC μl was added respectively to carry out the specific staining of CD4 molecules on the surface of the cell membrane, and then samples were incubated in their respective tubes at room temperature without light for 30 min. Then, 1 ml of PBS was added for another centrifugation (1500 r/min centrifugal for 5 min), and supernatant was discarded. 100 μl of Th1 or Th2 special cell fixative A (FIX&PERM A, Thermo Fisher Scientific, Waltham, MA, USA) was added, and the tubes were incubated at room temperature without light for 15 min. Other subsequent additions of 1ml of PBS followed by centrifugations (1200 r/min centrifugal for 5 min) and discard supernatant were undertaken accompanied with 100 μl Th1 or Th2 special cell fixative B (FIX&PERM B) and incubation at room temperature without light for 20 min, or incubation with 5 μl of anti-human γ-IFN-PE monoclonal antibody or IL-4-PE monoclonal antibody were also added into tubes to stain γ-IFN or IL-4 in cells, or 5 μl anti-mouse PE-anti-IgG1 antibody and tubes were incubated at room temperature without light for 20 min. The concentration of cells was adjusted to 10^5/ml to carry out flow analysis, and cellQuest analysis software was used to calculate the results.

ELISA Method: Kits were purchased from R&D Co. (Minneapolis, MN, USA) and used them in strict accordance with the steps in instructions.

Statistical Analysis

SPSS19.0 software (SPSS Inc., Chicago, IL, USA) was adopted to analyze all data; the mean ± standard deviation (SD) was used to represent measurement data; the comparison among groups were analyzed by one-way ANOVA; qualitative data was represented by a number or a percentage; inter-group comparison was tested by χ^2; $p<0.05$ indicated that difference was statistically significant.

Results

The Comparison of Th1/Th2

Th1 and Th1/Th2 of groups A and B were significantly lower ($p<0.05$) than those of groups C and D, but Th2 was significantly higher ($p<0.05$) than that of groups C and D. However, there was no difference between groups C and D (Table I and Chart 1).

The Comparison of the Level of hs-CRP and ISLP

The level of hs-CRP and TSLP of groups A and B was significantly higher ($p<0.05$) than that of groups C and D. There was no difference between groups C and D (Table II).

The Comparison of the Level of MCP-1 and Anti-Jo-1 Antibody

The level of MCP-1 and anti-Jo-1 antibody of groups A and B were significantly higher ($p<0.05$)

Table II. The comparison of the level of hs-CRP and ISLP.

Group	hs-CRP (mg/L)	TSLP (pg/mL)
Group A	15.6±3.3	452.6±62.4
Group B	10.3±3.1	387.9±53.5
Group C	4.7±1.5	123.4±34.2
Group D	4.2±1.6	115.6±30.8
F	9.527	12.624
p	<0.001	<0.001

Table III. The comparison of the level of MCP-1 and anti-Jo-1 antibody.

Group	MCP-1 (ng/mL)	Anti-Jo-1 antibody (U/mL)
Group A	5.62±1.05	234.8±42.6
Group B	4.78±1.21	210.6±35.9
Group C	1.30±0.34	46.8±12.3
Group D	1.12±0.26	42.9±13.6
F	8.457	16.629
p	<0.001	<0.001

than that of groups C and D. Whereas differences between groups A and B, groups C and D were similar (Table III).

Discussion

The mechanism of the occurrence of children's asthma may be a series of immune responses induced by T lymphocytes and mast cells and eosinophils. The interaction between dendritic cells (DCs) and external antigen is a critical step in the occurrence of allergic reactions[6]. DCs capture the external antigen and present it to the T lymphocytes of the pulmonary lymph nodes, and the response of T lymphocytes to antigen depends on the shape of antigen and the state of DCs. On the one side, if DCs are pre-stimulated by inflammatory signals, patterns recognition receptors change and then begin to take the initiative to identify the antigen to produce IL-12 factors as well as presenting the antigen to Th1 and secreting IL-2, INF-γ and other cytokines, which finally induces immune tolerance[7]. On the other side, if DCs fail to produce IL-12 signals, then Th2 cell differentiates and responses, secreting IL-4, IL-5, IL-13 and other cytokines[8]. The immunological memory, Th2, will produce a series of immune responses when confronted with the same antigen again, which manifests as allergic reactions. Therefore, the occurrence of asthma is probably because of the broken balance between Th1 and Th2, showing the reduction of Th1 and the increase of the Th2[9]. We can learn from the study that: Th1/Th2 of groups A and B was significantly lower than that of groups C and D, Th2 was significantly higher than that of group C and D, the level of hs-CPR and ISLP was significantly higher than that of groups C and D. There were differences in the above index of the comparison between groups A and B, but no difference between groups C and D. It suggests that the change of the level of Th1/Th2, hs-CRP and TSLP has a closer relationship with asthma than with ILD, the degree of change of the above index will increase when both exist compared with the pure asthma, it's not clear whether ILD plays a synergistic action[10]. ILD lesions involve alveolar septa, bronchial, vascular, interstitial lung air cavity around the tip, alveoli and bronchioles cavity, etc., the cause of ILD, clinical performance and the treatment effects of children are different from those of adults[11]. The anti-Jo-1 antibody is an anti-amino acid-tRNA synthetase antibody, which is regarded as a marked antibody of ILD. The anti-Jo-1 antibody can participate in the formation of lung immune complexes and trigger the lung macrophage activity, release chemokines and raise other inflammatory response components, which will result in lung injury[12]. MCP-1, as a member of the C-C subfamily of chemokines, owns monocyte chemotactic activity, in ILD lung tissue, the expression of MCP-1 is in epithelial cells, macrophages and vascular endothelial cells, which plays an important role in the inflammatory process of monocyte-macrophage cell mediation[13]. From this investigation, we can conclude that the level of MCP-1 and anti-Jo-1 antibody of group A and B were significantly higher than those of group C and D, the difference was statistically significant; there were differences of the comparison between groups A and B, but no difference between groups C and D.

In the clinical treatment, patients with the status of asthmatics combined with ILD are given with appropriate hormone programs with the efficiency rate of about 50-75%, when the symptoms of asthma relieve, the degree of lung injury will also significantly reduce[14]. It suggests that there may be a common or similar pathogenesis in the immune injury[15].

Conclusions

We showed that children's status asthmatics might have something to do with the abnormal expression of Th1/Th2, hs-CRP, TSLP, MCP-1 and anti-Jo-1 antibody, which provides references to further exploration of internal mechanism and therapeutic target of the two diseases.

Conflicts of interest

The authors declare no conflicts of interest.

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