Function Changes of Dendritic Cells in Peripheral Blood of Children with Bronchial Asthma

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ABSTRACT Objective: To investigate the function changes dendritic cells (DCs) from peripheral blood mononuclear cells (PBMC) in bronchial asthmatic children. Methods: PBMC-derived immature DCs(iDCs) were obtained from peripherial blood samples in 16 asthmatic patients and in 18 healthy children. iDCs were stimulated with rhTNF-α as mature DCs. The expression of the co-stimulatory molecules CD80(B7-1), CD86(B7-2) and CD83 were detected by fluorescent activated cell sorter and the levels of IL-10 and IL-12 in the supernatant were determined by enzyme-linked immunosobent assay. Results: ①The expression of CD86 on DCs was significantly higher than that of healthy control group ($t=2.27$, $P < 0.05$). There was no significant difference in the expression of CD80, CD83 between the two groups($t=1.17, 1.34$; $P > 0.05$). ②The level of IL-10 and IL-12 produced from DCs of asthmatic group were significantly lower than that of healthy control group ($t'=3.31, 3.39$; $P < 0.01$). ③IL-10 had postively relationship with IL-12 in asthma group ($r=0.740$, $P<0.01$), while there was no correlation in healthy control group ($r=0.232$, $P > 0.05$). Conclusion: DCs dysfunction existed in asthmatic children. It mainly manifested in the increase of the expression of CD86 and the decrease of the secretion of IL-10 and IL-12.

Key words: Asthma; Dendritic cell; Co-stimulatory molecules; Interleukin; Children

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Introduction

Bronchial asthma is a chronic inflame disease of airway induced by interactive actions of inflammatory cells and mediators. The pathogenesis is very complicated involved in many kinds of cells, inflammatory reaction mediators and cytokines, with characteristics of disbalance of Th1/Th2, advantage differentiation of Th2 cell and increase of cytokines secreted by Th2 cells such as IL-4, IL-5, IL-10, IL-13 [1]. This is also confirmed by animal experiments[2]. Promoting the proliferation of naive T cell, regulating Th1/Th2 reaction and playing an important role in immune response are the most significant characteristics of dendritic cell (DC), which is the most powerful APC discovered by now. The phenotype and secretion of cytokine was investigated and the role of DC in bronchial asthma was explored in the present study by culturing in vitro of DC.

1 Materials and Methods

1.1 Study subjects

1.1.1 Asthma group 16 asthma patients (10 males and 6 females) aged 3～10 yrs (average age:5.9 yrs) and hospitalized into pediatrics department from Feb. 2009 to Nov. 2009 were selected as patient group. Patients had been treated with glucocorticoids, immunopotentiators or immunodepressant orally or intravenously in past 4 weeks before blood collection were excluded.

1.1.2 Control group 18 healthy children (12 males and 6 females) , aged 2～11 yrs (average age: 7.3 yrs) , who had taken medical examination in well baby clinic of our hospital in the corresponding time period, were selected as control group. Children with recent infection and anaphylactic disease, or had been treated with immunodepressant were excluded.

1.2 Study norms and methods

1.2.1 Sample collection 3～4ml blood was acquired from peripheral vein in subjects on an empty stomach. Then the blood was reserved in sterile tube with heparin (20U/ml) for anticoagulation.

1.2.2 Peripheral blood mononuclear cell (PBMC) isolation PBMC was acquired by density gradient centrifugation, and the concentration of mononuclear cell suspension was adjusted to 2×10^6/ml with RPMI-1640 culture solution without cytokine (containing 10% inactivated fetal calf serum).

1.2.3 Induction and culture of DC 1ml PBMC suspension was poured into the well of 24-well cell culture plate, and incubated 3h under 37℃, saturated humidity, 5%CO_2. Non-adherent cells were removed after plate being washed softly by RPMI-1640 culture solution preheated under 37℃, and then adherent mononuclear cells were acquired. Then 1ml RPMI-1640 complete culture solution containing cytokine (the final concentration of rhGM-CSF and rh-IL-4 were 1000μ/ml and 500μ/ml respectively) was added. Then the cells were incubated for 8 days under 37℃, saturated humidity and 5%CO_2, and the growing state were detected everyday by using inverted microscope. Cytokines were added (the first dose halved) and semi-quantity change of liquid were performed every two days. rhTNF-a was added into all wells to the concentration of 200μ/ml on the sixth day. On the eighth day, the cells were ac-

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quired and centrifugated for 2 min under 10000rpm. Then the supernatant was collected and stored in refrigerator under -80℃. The redundant cells were adjusted to a proper concentration for the measurement of expression rate of CD80, CD83 and CD86 on the DC surface.

1.2.4 Phenotype analysis of DC DC acquired from the 2 groups was washed twice with phosphate buffer (DC), and then mixed after discarding supernatant. Then PBS was added to prepare the 1×10^5/ml cell suspension. Two group of 100 μl cell suspension were added into flow cytometer respectively, and then 20μl mouse anti-human CD80/83/86 monoclonal antibody, mice IgGI antibody (homeotype control of CD80 and CD83) and mice IgG2b antibody (homeotype control of CD86) marked by PE were also added respectively. Then the mixed solutions were incubated under 4℃ for 30 min and washed twice with PBS pre-cooled. At last, the solutions were analyzed again using flow cytometer after clarification.

1.2.5 Determination of culture suspension L−10 and IL−12 ELISA double antibody sandwich method was performed according to instruction of user's guide.

1.3 Statistical analysis

Statistical analysis was performed using SPSS17.0. Data with normal distribution was expressed as $\bar{x}\pm s$. t test and t' test were performed for analysis. Taking $P<0.05$ as statistical different and $P<0.01$ as significant statistical different.

2 Results

2.1 Morphological characteristics of DC during culture in vitro

Adherent cells were acquired from PBMC after 3h incubation. After 24h inducing culturing by cytokines of rhGM-CSF and rh-lL-4, adherent cells were decreased. After incubated for 3 days, apophysises with dendron appearance were present in cell bodies and the cells were aggregated. Then the number of suspension cells was increased and cell bodies were swelled. Cells were spread uniformly in culture medium after 7 days. Plenty of suspension cells with dendron appearance were present after 8 days.

2.2 Expression rate of costimulatory molecules on DC surface in children with asthma

There was no significant difference in expression rates of CD83 ($t=1.17$, $P>0.05$) and CD80 ($t=1.34$, $P>0.05$) on DC surface between children with asthma and healthy controls; the expression rate of CD86 on DC surface in asthma group was greater than that in health control group significantly ($t=2.27$, $P<0.05$)(Table 1).

Table 1 Analysis on DC phenotype acquired from PBMC in asthma group and control group($\bar{x}\pm s$,%)

Group	Cases(n)	CD83	CD80	CD86
Control group	18	45.88±13.10	14.84±7.57	23.92±6.24
Asthma group	16	41.00±13.36	18.11±6.52	30.12±9.49
t		1.17	1.34	2.27
P		>0.05	>0.05	<0.05

2.3 Change in cytokine lever excreted by DC in children with asthma

The IL-10 and IL-12 lever in suspension of DC culture solution of children with asthma were lower than that of health control group significantly ($t'=3.31$ or 3.39, $P<0.01$). The IL-12 lever in suspension of DC culture solution of children with asthma was positive correlated with IL-10 ($r=0.740$, $P<0.01$), there was no correlation in health control group ($r=0.232$, $P>0.05$)(Table 2).

Table 2 Comparison of cytokine lever excreted by DC acquired from PBMC in asthma group and control group ($\bar{x}\pm s$,ng/L)

Group	Cases(n)	IL-10	IL-12
Control group	18	363.27±57.95	88.63±5.48
Asthma group	16	312.23±25.53	75.12±13.62
t'		3.39	3.31
P		<0.01	<0.01

3 Discussions

CD83 is the indicator of DC maturation. Results from the present study showed that there was no significant difference in the expression of CD 83 on DC surface derived from PBMC between children with asthma and healthy children, which indicated that the maturation of CD in peripheral blood of children with asthma was normal. The relationship of pathway of costimulatory molecules CD80/CD86-CD28 and asthma was also confirmed in this study. It was showed that the Th2 reaction could not be induced by DC

in airway when T cell surface molecule CD28 was deficient (the ligand of CD80 and CD86). Blocking the interaction of CD80 /CD86 and ligand CD28 would suppress the gene expression of Th2 type cytokine[3]. The investigation on bronchial lavage fluid in asthma patients showed the activation and proliferation of T cell and the produce of IL-5 were dependent on CD86 [4]. Otherwise, the IgE-and IgG- antibody, eosinophil in airway and airway hyperreactivity were inhibited significantly when the mouse treated by anti-CD86 (not anti-80) exposed in ovalbumin aerosol[5]. In conclusion, the CD86 play a more important role than CD80 in the induction of Th2 response. Compared with health control group, the expression of costimulatory molecules 86 in DC derived from PBMC in asthma group was increased significantly, but the change in CD80 was not significant, which indicating that when expression of CD86 was up-regulated by DC, the CD 28 in T cell would be induced to activate the differentiation of primitive T cell to Th2, and then the whole chain reaction led to asthma. This was also confirmed in animal experiment[6].

IL-12 produced by APC, especially by DC, was the key factor influencing the balance of Thl/Th2. On the one hand, it activated Th1 response and made Th1 producing IFN-γ continually, and the latter could enhance the expression of IL-12 in turn; on the other hand, it could inhibit Th2 type response and the synthesis of Th2 type cytokine directly to suppress the over-synthesis of IgE by lymphocyte B. The immune reaction would prone to the direction of Th2 when the expression of IL-12 was deficient, and the Ⅰ type allergy mediated by IgE would be induced by allergen or viruses, which eventually resulted in asthma. Just as the results confirmed by MAO Guangyu et al, the IL-12 lever secreted by DC in asthma group in the present study was lower than in that of health control group significantly[7]. Nasser et al found that the expression lever of IL-12 mRNA in respiratory tract of allergic asthma patients were lower than that of health adult [8]. Some other researches showed that the effect of histamine on phenotype change of mature DC drove by LPS and polarization of naive T cell was insignificant, but the types of cytokine and chemokine excreted by mature DC could be changed by histamine to a great degree. Especially when the H2 receptor was affected by histamine temporally, the production of IL-10 and decrease of IL-12 excretion would eventually lead to the differentiation of native CD4+T cell to Th2 under the induction of mature DC [9]. The mechanism of the decrease of IL-12 was not clear yet, might be: ① there was some defection in asthma patients' DC, which suppressed the excretion of IL-12. ②high proportion lymphoid DC (DC2) was constructive expressed in asthma patient due to the changed balance of DC in different subgroups, but the production of IL-12 was less than in myeloid DC (DC1) [10]. ③ the excretion of IL-12 was suppressed by cytokines with down regulation, such as IL-10, histamine, and PGE2 etc, through acceptor mediation.

In the early stage, IL-10 was recognized as Th2 type cytokine which playing an important role in the differentiation and development of Th0 to Th2. Later, researchers found that IL-10 could be produced by plenty of cells, such as regulatory T cell (Trl cell), helper T cell (such as Th0 and Th2), B cell, mononuclear macrophage and mastocyte etc. Currently, more and more researchers presumed it to be a protective cytokine. Otherwise, the specific tolerance of antigen in respiratory tract could also be induced by IL-10, during which the allergy was inhibited through the depression on T cell response. It was confirmed by some investigation that the Th0 did not differentiate to Th2 but to regulatory T cell under induction of IL-10 released from DC, the later one expressed a high lever of IL-10, leading to the cell tolerance Th and decreased response of Th2 [11]. The decrease of IL-10 produced by DC acquired from PBMC in asthma patients was confirmed in this study, indicating that asthma could be induced by the dysfunction of DC and decrease of IL-10, together with the deficient of Th tolerance and enhanced Th2 response. The negative regulation effect of IL-10 could not be excluded neither.

There were some researches showed that the synthesis of IL-12 by DC could be suppressed by IL-10 [12-13]. The present study showed that there was a positive correlation between the synthesis of IL-12 and IL-10 in asthma patients but not seen in health, which was contradict previous results. The reason might be that the synthesis of IL-12 was not only regulated by IL-10, but also by many other factors, such as antigenic information, IFN-γ , individual genetic characteristic etc[14]. The exact mechanism must be investigated in further study.

One in word, asthma was induced by plenty of factors together. Due to the dysfunction of DC, the expression of CD86 was increased and synthesis of IL-12 and IL-10 was decreased, which made the balance of Th1/Th2 being prone to the direction of Th2, and eventually asthma was induced. If the phenotype and dysfunction of DC could be investigated further, DC might become a new target position for the treatment of asthma.

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支气管哮喘病儿外周血树突状细胞功能变化

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摘要 目的:观察支气管哮喘病儿外周血单个核细胞(PBMC)来源树突状细胞(DC)功能变化。方法:以18例健康儿童为对照,选择16例支气管哮喘发作期病儿为研究对象,分离PBMC并经rhGM-CSF诱生成熟DC。采用流式细胞仪(FACS)检测DC表面共刺激分子CD80(B7-l)、CD86(B7-2)和CD83的表达率;ELISA法检测培养上清液中IL-10和IL-12的变化。结果:①哮喘组DC表面CD86的表达率明显高于健康对照组(t=2.27,P<0.05),CD80、CD83的表达率与健康对照组比较均无显著性差异(t=1.17,1.34;P>0.05)。②哮喘组DC分泌IL-10、IL-12水平均明显低于健康对照组(t'=3.31,3.39;P<0.01)。③哮喘组DC分泌IL-10与IL-12成正相关(r=0.740,P<0.01),而健康对照组IL-10与IL-12无相关性(r=0.232,P>0.05)。结论:支气管哮喘病儿DC存在功能缺陷,主要表现在CD86表达升高、IL-10、IL-12分泌减少。

关键词:支气管哮喘;树突状细胞;共刺激分子;白细胞介素;儿童

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