Int J Clin Exp Med 2017;10(1):1484-1489
www.ijcem.com /ISSN:1940-5901/IJCEM0037186

Original Article

T follicular helper cells in peripheral blood are associated with the expression of B cells and plasma IgE level in patients with acute asthma exacerbation

Yi Hu[1*], Sunfeng Yu[1*], Jinrong He[2], Su Zhao[1], Shuang Geng[1]

[1]Department of Respiratory Medicine, [2]Key Laboratory for Molecular Diagnosis of Hubei Province, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China. [*]Equal contributors.

Received August 3, 2016; Accepted September 20, 2016; Epub January 15, 2017; Published January 30, 2017

Abstract: Differentiation of T follicular helper (Tfh) cells was enhanced in patients with acute asthma exacerbation, but their associations with the expression of B cells and IgE level were not clear. In this study, we aimed to explore the correlations between Tfh cells and B cells, as well as plasma IgE, IgG_1 levels. Peripheral blood samples were collected from forty-two patients with acute exacerbation and forty-eight matched controls. Tfh cells ($CD4^+CXCR5^+$) and B cells ($CD19^+$) were examined by flow cytometry, while plasma IgE, IgG_1 and IL-21 level were measured by enzyme-linked immunosorbent assay (ELISA). The percentage of Tfh cells ($P<0.001$), B cells ($P<0.001$) in peripheral blood, plasma IgE ($P<0.001$), IgG_1 ($P<0.001$) and IL-21 ($P<0.001$) levels were all increased in patients with acute asthma exacerbation compared with health controls. The percentage of Tfh cells was associated with B cells ($r=0.514$, $P<0.001$) and plasma IgE level ($r=0.620$, $P<0.001$), while no significant correlation between the percentage of Tfh Cells and IgG_1 ($r=0.255$, $P=0.103$) in asthma patients with acute exacerbation was observed. Similarly, plasma IL-21 level was associated with B cells ($r=0.831$, $P<0.001$) plasma IgE level ($r=0.324$, $P=0.036$), with no significant correlation between the plasma level of IL-21 and IgG_1 ($r=0.207$, $P=0.188$) in asthma patients with acute exacerbation. Tfh cells may participate in the airway inflammation reaction through promoting the differentiation and IgE class switching of B cells in asthma patients with acute exacerbation.

Keywords: T follicular helper cells, B cells, plasma IgE, acute asthma exacerbation

Introduction

T follicular helper cells (Tfh cells) are the major cells which could induce the differentiation, proliferation and activation of B cells and help the class switching of the immunoglobulin antibody [1, 2]. It has been proved that both the differentiation and secretion function of Tfh cells would be enhanced in acute bronchial asthma (Hereinafter referred to as "asthma") exacerbation [3, 4], but it still remained unclear whether it mediated the differentiation and secretion function of B cells in asthma. This study aimed to evaluate the differences of the molecular mark on the surface of Tfh cells ($CD4^+CXCR5^+$), the level of its primary secretion cytokines (IL-21), the molecular mark on the surface of B cells ($CD19^+$) as well as plasma IgE and IgG_1 between patients with acute asthma exacerbation and healthy volunteers, and to analyze the correlations of Tfh cells, IL-21 level with B cells, plasma IgE and IgG_1 levels in peripheral blood, to investigate whether the enhancement of Tfh cells differentiation participated in the differentiation and secretion of B cells, which could lay the foundation for further study in the mechanism of Tfh cells in asthma.

Methods

Participants

42 patients with asthma from Department of Respiratory Medicine in the Central Hospital of Wuhan between June 2013 and May 2015 were included. Inclusion criteria: matched the classification of asthma of the American Thoracic Society criteria [5], patients with clinical symptoms of typical acute asthma exacerba-

Table 1. Characteristics of patients with acute asthma exacerbation and controls

Parameters	Patients with acute asthma exacerbation (n=42)	Controls (n=48)	*p* Value
Age (years)	41±10	44±11	0.319
Female (%)	22 (52%)	23 (48%)	0.673
FVC (L)	2.1±0.3	3.2±0.2	P<0.001
FEV_1 (L)	1.3±0.2	2.6±0.2	P<0.001
FEV_1/FVC (ratio %)	60.7±2.9	82.5±3.2	P<0.001

Data are presented as Mean values ± SD or count and percentages. Significance of the comparison is determined by the Student t test and the χ^2 test. FVC, forced vital capacity; FEV_1, forced expiratory volume in one second.

tion (including asthma, chest distress, dyspnea and increased cough), bronchial provocation or positive result in diastolic test, positive result in allergy skin test, increased plasma IgE level and increased eosinophilic granulocyte in peripheral blood. Exclusion criteria: patients with the history of immune or tumor diseases, or patients who had acute infection within 4 weeks or received general or cortical hormone therapy in recent 4 weeks.

48 healthy volunteers from physical examination department of the Central Hospital of Wuhan were included as controls. Inclusion criteria: no history of asthma, non-allergic constitution, negative results in allergy skin test, normal lung function, no history of respiratory disease, no history of immune or tumor diseases, no acute infection within 4 weeks.

All the participants agreed to participate in this study and signed the written informed consent. This clinical protocol has been approved by the Ethics committee of the Central Hospital of Wuhan.

Blood sample

The peripheral blood samples from patients with acute asthma exacerbation were collected before the treatment, the peripheral blood samples from healthy volunteers in control group were collected during the physical examination.

Identification of Tfh cells and B cells levels in peripheral blood by flow cytometry

5~8 ml venous blood from every subject was collected by heparin sodium anticoagulation pipets, and centrifuged for 8 min in 800 r/min at room temperature, the supernatant was taken and cryopreserved in -80°C fridge. Then it was diluted by PBS, the peripheral blood mononuclear cells (PBMCs) were separated according to the instruction of Ficoll Lymphocyte separation medium (Nycomed company, Norway), the washed cells were collected, divided into two groups and added with anti-human CD4-PE, CXCR5-FITC, CD19-PE antibody (all from eBioscience company, USA), the mice IgG marked by FITC and the rabbit IgG_{2a} marked by PE were Isotype controls. Incubated for 40 min away from light, washed twice by PBS, after fixed with fixative (eBioscience company, USA), tested the expression proportion of the molecular mark on the surface of the cells by BD FACSAriaTM flow cytometry. Expression proportion of $CD4^+$ $CXCR5^+$ cells (Tfh cells) and $CD19^+$ cells (B cells) was determined by lymphoid cells in PBMCs.

Identification of the plasma IL-21, IgE and IgG_1 levels in peripheral blood by Elisa

The peripheral blood plasma from the subjects was collected and cryopreserved, the IL-21, IgE and IgG_1 levels were examined by Elisa according to the method described in the instruction (all from eBioscience company, USA). The detection range of IL-21 was 8~1000 pg/mL, the detection range of IgE was 7.8~1000 ng/ml, the detection range of IgG_1 was 0.16~10 μg/ml. All the samples were tested for three times repeatedly.

Statistical analysis

Data was presented as mean values ± SD, median and 25th-75th quartile or percentages. The variation analysis was conducted by Student t, Mann-Whitney or χ^2 test; Pearson and Spearman test were used for correlation analysis. All the statistical analysis was conducted using SAS 8.1, P<0.05 was defined as statistical significance.

Results

Characteristics of patients with acute asthma exacerbation and health controls

There was no significant difference between the age (41±10 years old) and gender (female

Table 2. Tfh cells ($CD4^+CXCR5^+$) and B cells ($CD19^+$) in patients with acute asthma exacerbation and controls

Marker	Patients with acute asthma exacerbation (n=42)	Controls (n=48)	*p* Value
$CD4^+CXCR5^+$	22.53%±5.40%	17.21%±4.52%	<0.001
$CD19^+$	6.79%±2.57%	3.05%±0.69%	<0.001

Data are presented as Mean values ± SD. Significance of the comparison is determined by the student t test.

Table 3. Plasma levels of IgE, IgG_1, IL-21 in patients with acute asthma exacerbation and controls

Parameters	Patients with acute asthma exacerbation (n=42)	Controls (n=48)	*p* Value
IgE (ng/ml)	88.42 (39.40-172.93)	39.65 (18.54-68.72)	<0.001
IgG_1 (ug/ml)	0.908 (0.473-1.217)	0.542 (0.342-0.813)	<0.001
IL-21 (pg/ml)	181.61 (68.21-221.67)	78.42 (33.49-136.11)	<0.001

Data are presented as median and 25th-75th quartile. Significance of the comparison is determined by the Mann-Whitney test.

22/52%) of the patients with acute asthma exacerbation and the health controls (age 44± 11 years old, female 23/48%), P=0.319 and P=0.673, respectively. As shown in **Table 1**.

Tfh and B cells ratio, plasma IL-21, IgE and IgG_1 levels in patients with acute asthma exacerbation

$CD4^+CXCR5^+$ cells (Tfh cells) proportion and $CD19^+$ cells (B cells) proportion in the lymphocyte of patients with acute asthma exacerbation were significantly higher than that in health controls (22.53%±5.40% vs. 17.21%± 4.52%, P<0.001; 6.79%±2.57% vs. 3.05%± 0.69%, P<0.001), presented in **Table 2**. Besides, plasma levels of IL-21, IgE, IgG_1 were remarkably elevated in asthma exacerbation patients compared with health controls [181.61 (68.21-221.67) vs. 78.42 (33.49-136.11), P< 0.001; 88.42 (39.40-172.93) vs. 39.65 (18.54-68.72), P<0.001; (0.908 (0.473-1.217) vs. 0.542 (0.342-0.813), P<0.001, respectively], as shown in **Table 3**.

Correlations analysis of Tfh ratio with B cells ratio, IgE and IgG_1 levels in patients with acute asthma exacerbation

$CD4^+CXCR5^+$ cells (Tfh cells) proportion and $CD19^+$ cells (B cells) proportion were positive correlated in patients with acute asthma exacerbation (r=0.514, P<0.001) (**Figure 1A**). Similarly, the Tfh cells proportion was positively correlated to the plasma IgE level in peripheral blood (r=0.620, P<0.001) (**Figure 1B**). However, Tfh cells proportion and plasma IgG_1 level in peripheral blood did not present any significant correlation (r=0.255, P=0.103) (**Figure 1C**).

Correlation analysis of the IL-21 level and B cells level, IgE, IgG_1 in peripheral blood plasma of patients with acute asthma exacerbation

Plasma IL-21 level in peripheral blood presented positive correlation with $CD19^+$ (B cells) in lymphocyte (r=0.831, P< 0.001) (**Figure 2A**) in patients with acute bronchial asthma exacerbation, as well as IgE level (r=0.324, P=0.036) (**Figure 2B**). However, plasma IL-21 and IgG_1 level did not show any significant correlation (r=0.207, P=0.188) (**Figure 2C**).

Discussion

In the pathogenesis of asthma, both the cellular immunity mediated by T helper cells and the humoral immunity mediated by B cells participates in the disease process [6, 7]. The humoral immunity induced by IgE and IgG_1 from B cells is the primary immunologic mechanism of athsma [8]. In the mice model of bronchial asthma, the formation of germinal center, activation of B cells and secretion of IgE could be observed in lymphoid organ and inflammatory lung tissue. Meanwhile, the activation, proliferation and differentiation of B cells are mediated by the T lymphocyte subpopulation: T Follicular helper (Tfh) cells [1, 2]. Currently, it has been demonstrated that the differentiation of Tfh cells is increased in the patients with acute asthma exacerbation [3, 4], but it is still not clear whether the enhancement of Tfh cells differentiation and secretion were associated with B cells.

IL-21 is the most important cytokine in secretion of Tfh cells, which contributes to the adaptive proliferation of B cells in germinal center [9,

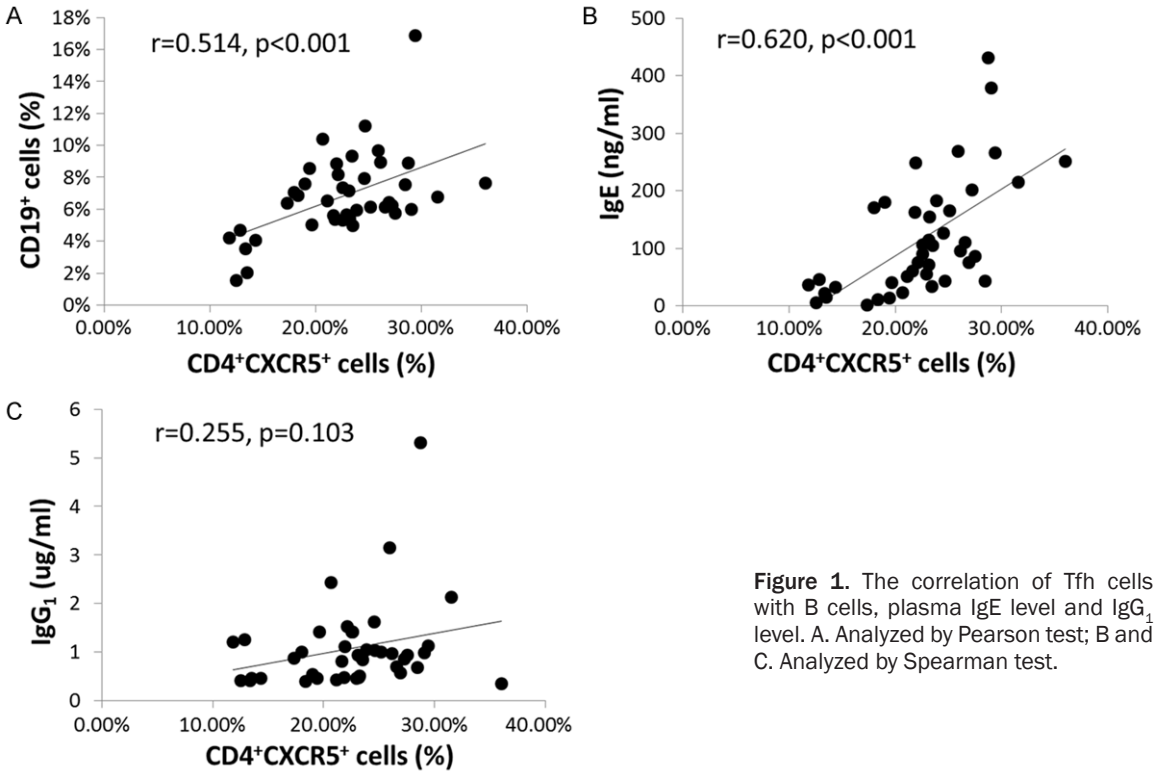

Figure 1. The correlation of Tfh cells with B cells, plasma IgE level and IgG_1 level. A. Analyzed by Pearson test; B and C. Analyzed by Spearman test.

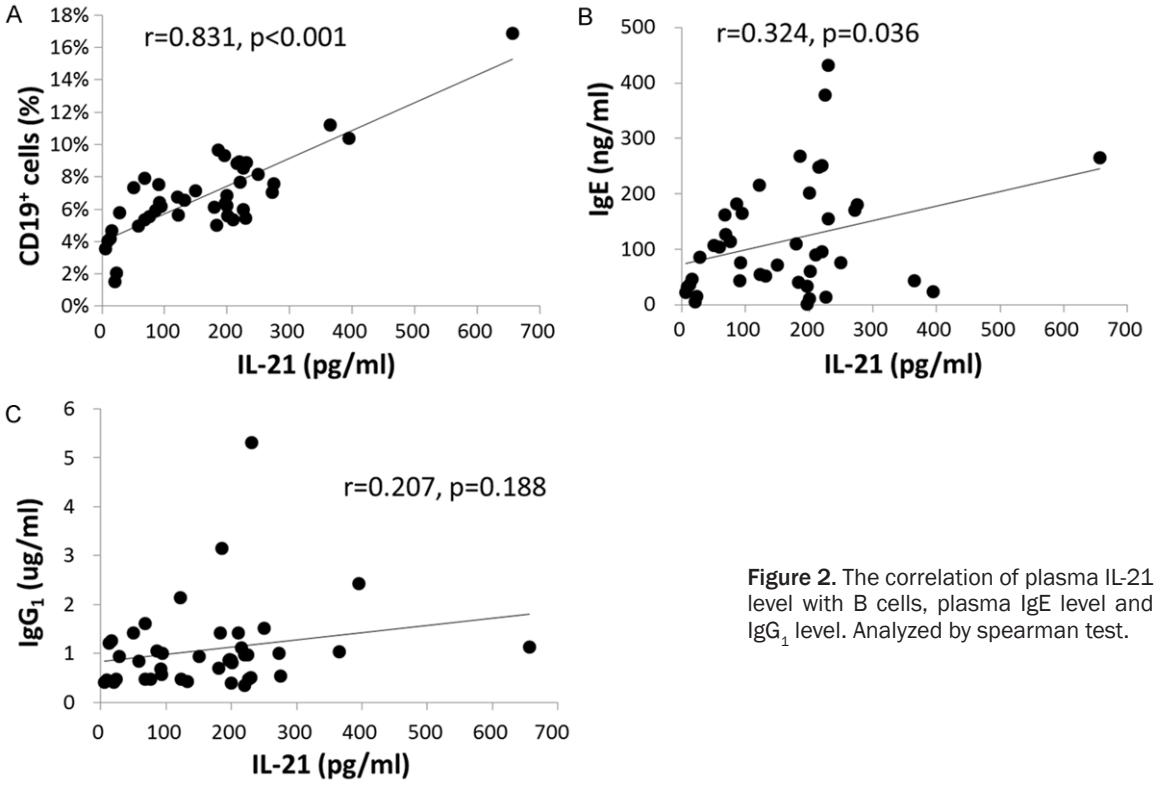

Figure 2. The correlation of plasma IL-21 level with B cells, plasma IgE level and IgG_1 level. Analyzed by spearman test.

10]. Both in vivo and in vitro test, IL-21 promotes the differentiation of the plasma cells via activating STAT3 [11-13], meanwhile increasing the IL-4 mediated transformation of IgE in

human B cells through the STAT3-dependent pathway [14]. In addition, IL-21 increases the Tfh cells proportion in vivo and vitro conversely, indicates the interaction between IL-21 and Tfh cells [15].

In this study, we presented that the proportion of Tfh cells in patients with acute asthma exacerbation and IL-21 level in peripheral blood plasma were positively correlated to B cells, which indicated that the differentiation of Tfh cells and increased secretion of IL-21 might contribute to the proliferation and differentiation of B cells during the onset of asthma. In patients with eosinophils nasal polyps, the number of Tfh cells presents positive correlation with the maturity of B cells in germinal center [16]. While the proportion of Tfh cells and IL-21 level in peripheral blood plasma in patients with acute asthma exacerbation were positively correlated to IgE level in peripheral blood plasma, which indicated that the differentiation of Tfh cells stimulated the class switching of IgE in B cells. In eosinophils nasal polyps, the investigators found that the proportion of IL-21$^+$ Tfh cells are positively related to IgE level in plasma [16].

IgG_1 level was increased in asthma alveolar lavage fluid [17, 18]. While in plasma, in our previous study, we have demonstrated that the IgG_1 level of patients with acute asthma exacerbation was apparently higher than that in health control group, and increased IgG_1 level was positively correlated to the enhancement of differentiation of B cells [19]. However, in present study, T cells in patients with acute asthma exacerbation and the IL-21 level in peripheral blood plasma showed no obvious correlation with the secretion of IgG_1 in peripheral blood plasma, which indicated that Tfh cells might not participate in the class switching of IgG_1. Early study found that the IgG_1 level is evaluated in children who were allergic to house dust compared with children without allergy, but the IgG_1 level in the acute exacerbation was lower than that in the remission status [20]. Another study on patients who were allergic to birch pollen also found that IgG_1 suppresses the anaphylaxis reaction. They believed that IgG_1 probably inhibits the combination between IgE and its target site through competing combination of itself, to suppress the allergy reaction [21]. So currently it is still controversial what role of IgG and its subtype in Th2 airway allergic reaction are, and it needs further study on whether there is any other mechanism in the finding showed in present study that there was no correlation between the differentiation of Tfh cells and IgG_1.

This study presented that Tfh cells was positively correlated to B cells and plasma IgE level in patients with acute asthma exacerbation through correlation analysis, as well as IL-21, which indicated that Tfh cells and IL-21 might promote the participation of class switching of IgE in airway inflammation in asthma via stimulating the proliferation of B cells. Next step, we will investigate in animal experiment whether the direct blocking of the differentiation of Tfh cells could affect the differentiation and function of B cells in asthma mice model.

Acknowledgements

This study was supported by National Natural Science Foundation of China (No. 81400020) and Scientific research project of Wuhan municipal health and Family Planning Commission (No. WX14A03).

Disclosure of conflict of interest

None.

Address correspondence to: Shuang Geng, Department of Respiratory Medicine, The Central Hospital of Wuhan, Tongji Medical College, Huazhong University of Science and Technology, No. 26, Shengli Road, Wuhan 430014, China. Tel: +86-027-82211466; Fax: +86-027-82211466; E-mail: gass08@163.com

References

[1] Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P and Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med 2000; 192: 1553-62.

[2] Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M and Forster R. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med 2000; 192: 1545-52.

[3] Gong F, Su Q, Jiang D, Chen J, Pan Y and Huang X. High frequency of circulating follicular helper T cells in patients with bronchial asthma. Clin Lab 2014; 60: 963-8.

[4] Geng S, Gao S, Hu H, Hu Y, Yu S and Zhao S. [The differentiation and clinical significance of follicular helper T cells during acute exacerbation in asthma patients]. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi 2015; 31: 1383-6.

[5] Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American thoracic society was adopted by the ATS board of directors, November 1986. Am Rev Respir Dis 1987; 136: 225-44.

[6] Lloyd CM and Hessel EM. Functions of T cells in asthma: more than just T(H)2 cells. Nat Rev Immunol 2010; 10: 838-48.

[7] Holgate ST and Polosa R. The mechanisms, diagnosis, and management of severe asthma in adults. Lancet 2006; 368: 780-93.

[8] Williams JW, Tjota MY and Sperling AI. The contribution of allergen-specific IgG to the development of th2-mediated airway inflammation. J Allergy (Cairo) 2012; 2012: 236075.

[9] Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, Verma NK, Smyth MJ, Rigby RJ and Vinuesa CG. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med 2010; 207: 353-63.

[10] Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, Corcoran LM, Godfrey DI, Toellner KM, Smyth MJ, Nutt SL and Tarlinton DM. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med 2010; 207: 365-78.

[11] Ozaki K, Spolski R, Ettinger R, Kim HP, Wang G, Qi CF, Hwu P, Shaffer DJ, Akilesh S, Roopenian DC, Morse HC 3rd, Lipsky PE and Leonard WJ. Regulation of B cell differentiation and plasma cell generation by IL-21, a novel inducer of Blimp-1 and Bcl-6. J Immunol 2004; 173: 5361-71.

[12] Bryant VL, Ma CS, Avery DT, Li Y, Good KL, Corcoran LM, de Waal Malefyt R and Tangye SG. Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5+ T follicular helper cells. J Immunol 2007; 179: 8180-90.

[13] Avery DT, Deenick EK, Ma CS, Suryani S, Simpson N, Chew GY, Chan TD, Palendira U, Bustamante J, Boisson-Dupuis S, Choo S, Bleasel KE, Peake J, King C, French MA, Engelhard D, Al-Hajjar S, Al-Muhsen S, Magdorf K, Roesler J, Arkwright PD, Hissaria P, Riminton DS, Wong M, Brink R, Fulcher DA, Casanova JL, Cook MC and Tangye SG. B cell-intrinsic signaling through IL-21 receptor and STAT3 is required for establishing long-lived antibody responses in humans. J Exp Med 2010; 207: 155-71.

[14] Avery DT, Ma CS, Bryant VL, Santner-Nanan B, Nanan R, Wong M, Fulcher DA, Cook MC and Tangye SG. STAT3 is required for IL-21-induced secretion of IgE from human naive B cells. Blood 2008; 112: 1784-93.

[15] Eto D, Lao C, DiToro D, Barnett B, Escobar TC, Kageyama R, Yusuf I and Crotty S. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS One 2011; 6: e17739.

[16] Zhang YN, Song J, Wang H, Wang H, Zeng M, Zhai GT, Ma J, Li ZY, Liao B, Wang BF, Zhen Z, Wang N, Cao PP, Lin P, Ning Q and Liu Z. Nasal IL-4(+)CXCR5(+)CD4(+) T follicular helper cell counts correlate with local IgE production in eosinophilic nasal polyps. J Allergy Clin Immunol 2016; 137: 462-73.

[17] Kitz R, Ahrens P and Zielen S. Immunoglobulin levels in bronchoalveolar lavage fluid of children with chronic chest disease. Pediatr Pulmonol 2000; 29: 443-51.

[18] Out TA, van de Graaf EA, van den Berg NJ and Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. Scand J Immunol 1991; 33: 719-27.

[19] Wang LC, et al. Level and clinical value of serum IgG1 in asthma patients [in Chinese]. Medical Journal of Wuhan University 2016; 37.

[20] Hales BJ, Martin AC, Pearce LJ, Laing IA, Hayden CM, Goldblatt J, Le Souef PN and Thomas WR. IgE and IgG anti-house dust mite specificities in allergic disease. J Allergy Clin Immunol 2006; 118: 361-7.

[21] Visco V, Dolecek C, Denepoux S, Le Mao J, Guret C, Rousset F, Guinnepain MT, Kraft D, Valenta R, Weyer A, Banchereau J and Labecque S. Human IgG monoclonal antibodies that modulate the binding of specific IgE to birch pollen Bet v 1. J Immunol 1996; 157: 956-62.