

B7-H3 expression in children with asthma exacerbation

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ABSTRACT

Background: Recent studies have shown that B7-H3, a recently identified B7 family member, plays a critical role in the development of asthma.

Objective: This study is to explore the expression of B7-H3 in children with asthma exacerbation.

Methods: Twenty-one Chinese children with asthma exacerbation as well as 18 nonasthmatic control Chinese children were enrolled. B7-H3 level and cytokines (interferon [IFN]- γ , interleukin [IL]-4, and IL-10) determination were performed by enzyme-linked immunosorbent assay (ELISA) technique. Meanwhile, clinical parameters including laboratory findings, forced expiratory volume in one second (FEV₁) and fractional exhaled nitric oxide were obtained.

Results: Children with asthma exacerbation had significantly higher levels of B7-H3 than controls (4.46 ± 1.33 versus 3.42 ± 1.48 ng/mL; $p = 0.027$). Plasma IL-4 level was significantly higher in asthma exacerbation subjects than controls (157.98 ± 21.57 versus 121.92 ± 24.37 pg/mL; $p < 0.0001$), and IFN- γ level was significantly lower in asthma exacerbation subjects (292.73 ± 152.47 versus 421.78 ± 145.84 pg/mL; $p = 0.0107$). Level of B7-H3 in asthma exacerbation subjects with inhaled corticosteroid (ICS) treatment recently was significantly lower than subjects without ICS treatment ($t = 2.706$; $p = 0.0136$). Additionally, levels of B7-H3 decreased remarkably after prednisone treatment. Level of sB7-H3 in asthma exacerbation subjects was inversely correlated with level of IFN- γ ($r_p = -0.605$; $p = 0.005$) after adjustment.

Conclusion: B7-H3 may play an important role in asthma exacerbation and was a useful clinical biomarker to evaluate asthma exacerbation.

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The incidence of allergic diseases, including bronchial asthma, has dramatically increased in recent decades, affecting an estimated 235 million people worldwide and the burden is likely to rise substantially in the next few decades. Allergic asthma is a disease characterized by chronic airway inflammation with infiltration of eosinophils, increased mucus production in the bronchioles, and airway hyperreactivity (AHR) to a variety of specific and nonspecific stimuli.

A variety of cell types are involved in allergic inflammation such as eosinophils, mast cells, and T-helper 2 (Th2) cells, which breaks the fine balance between Th1 and Th2 cells toward Th2 bias. It is well known that Th2 cells are common in the lungs of allergic asthmatic subjects with increased levels of Th2 cytokines as interleukin (IL)-4, IL-5, and IL-13,^{1,2} which could stimu-

late basophils and eosinophils and enhance mast cell differentiation^{3–5} as well as Th1 cells are important in defense against viruses and bacteria by producing IL-2 and interferon (IFN)- γ ,⁶ which may also protect from allergic polysensitization in children.⁷ IL-10 produced by T regulatory (Treg) cells was suggested to play a role in suppressing Th2 responses to allergens.⁸

Consequently, much of the ongoing basic research in asthma is directed at understanding how Th2 cytokines cause asthma-like pathology and physiology,⁹ but the molecular mechanisms for the overproduction of Th2 cytokines or decreasing Th1 and Treg cytokines in asthmatic responses have not been fully understood. The identification and development of biomarkers for distinct molecule of Th cell cytokines in asthmatic responses would guide the direction of basic research and the clinical application of emerging asthma therapies that specifically target Th cell responses in the lung.

Optimal activation of antigen (AG)-specific T cells requires engagement of the T-cell receptor with Ag/major histocompatibility complex and a costimulatory signal provided by antigen presenting cells.¹⁰ It is well known that cluster of differentiation (CD)28-B7 (B7-1 and B7-2) interaction plays a major role in providing a costimulatory signal for T cells.¹¹ Blockade of these costimulatory pathways markedly reduced allergen-induced asthma in mice,¹² suggesting a promising approach for the treatment of allergic disease in humans.

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B7-H3, a newly discovered member of the B7 superfamily, has been identified in both humans and mice by sharing approximately 88% amino acid sequence identity.¹³ B7-H3 is not expressed in significant amounts on freshly isolated human lymphocytes but is induced in human monocytes/macrophages and dendritic cells upon inflammatory cytokine stimulation. The receptor for B7-H3 has not yet been identified, but it is thought to be expressed by activated T cells and is apparently distinct from other known T-cell costimulatory receptors.^{14,15} Initial studies showed that human B7-H3 could augment Ag-specific T-cell receptor-mediated T-cell proliferation, enhance the induction of cytotoxic T cells, and selectively stimulate IFN- γ production.¹³ In contrast, other groups have reported a negative regulatory function for both human and mouse B7-H3 in T cell-mediated immune responses, including an inhibitory effect of B7-H3 on anti-CD3 monoclonal antibody (mAb)-induced both T-cell proliferation and cytokine production.^{14–16} Accumulated evidence above supports the notion that B7-H3 has a contrasting role in regulating T cell-mediated immune responses by functioning as both a T-cell costimulator and coinhibitor.

Blockage of B7-H3 could alleviate the asthmatic syndrome, decreased the levels of B7-H3 positive cells in the lung tissues, abrogated hypercellularity, eosinophil infiltration, and mucus production, and inhibited IL-4 and IL-17 production in bronchoalveolar lavage fluid as described previously.¹⁷ Using newly generated antibody that specifically bind B7-H3 with high affinity, we developed a quantitative enzyme-linked immunosorbent assay (ELISA) kit for the measurement of soluble B7-H3. We previously reported that soluble B7-H3 exists in human plasma and is a functionally active form and human sB7-H3 was cleaved by some matrix metalloproteinase from the surface of activated T lymphocytes, monocytes, and monocyte-derived dendritic cells.¹⁸ The objective of the present study was to explore the relationships between sB7-H3 and levels of distinctive Th1, Th2, and Treg cytokines in allergic asthma children and subsequently analyze the usefulness of sB7-H3 level in plasma for prognostic evaluation of children with asthma.

METHODS

Study Design

This study included 21 Chinese children with moderate to severe asthma exacerbation as well as 18 Chinese non-asthmatic control children in the age group of 5–12 years. The diagnosis of asthma and its severity of asthma exacerbation were done according to Global Initiative for Asthma guidelines.¹⁹ Exacerbation is an acute or sub-acute worsening in symptoms and lung function from the patients' usual status and all the children included in this study had the symptoms of wheezing, dyspnea, and tachypnea after treatment with inhaled salbutamol. Chil-

dren with moderate to severe asthma exacerbation were recruited from the Department of Respiratory Disease in Children's Hospital Affiliated to Soochow University (Suzhou, China), whereas the controls were chosen randomly from surgery wards without history of lung disease, wheezing, allergy, AHR and respiratory infections and no significance of age and gender was found. This study was conducted with the approval of the Institutional Human Ethical Committee of the Soochow University. An informed consent was obtained from all the subjects or guardians who participated in this study.

Upon hospital admission of the patients, pediatricians completed a questionnaire regarding demographic data of the patient, the date of onset of the disease, the clinical symptoms, the laboratory findings as well as forced expiratory volume in one second (FEV₁) and fractional exhaled nitric oxide, which were conducted by a professional staff using spirometric assessment (MasterScope; JAEGER, Hoechberg, Germany) and the portable multigas analyzer (NIOX MINO, Aerocrine, Sweden) in accordance with published guidelines.^{20,21} Meanwhile, each control or asthmatic child's plasma (4 mL) was obtained for detecting eosinophils, CD19⁺CD23⁺ B lymphocytes, levels of IFN- γ , IL-4, IL-10, and sB7-H3 in peripheral blood. Allergen-specific immunoglobulin E detection using Pharmacia CAP System (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden) and skin-prick tests were applied to all subjects for confirming sensitization to allergens. As for excluding asthma exacerbation due to respiratory infection such as virus and mycoplasma pneumoniae, nasopharyngeal aspiration fluids were collected from all of the subjects within 24 hours of admission for multipathogen detection as previously described.²²

Flow Cytometric Analysis

The peripheral blood of asthma patients and healthy donors were obtained and peripheral blood mononuclear cells (PBMCs) were isolated and stained with fluorescein isothiocyanate-B7-H3 (red lines) or biotinylated human IgG (blue lines) as the control and phycoerythrin-CD3, CD14, CD19, and CD56 (eBioscience, San Diego, CA) and were analyzed by using a FAC-Scanto flow cytometer and Diva software (BD Biosciences, San Jose, CA). The anti-B7-H3 mAbs (21D4) were identified and developed in our laboratory.¹⁸

Examination of Plasma Levels of IFN- γ , IL-4, IL-10, and sB7-H3

Samples of plasma for cytokines and sB7-H3 detection were obtained from children at the time of admission and discharge. The samples were immediately centrifuged and preserved at -80°C for subsequent assays. All the cytokines were detected through ELISA

Table 1 Demographic data and clinical characteristics of asthma exacerbation and control subjects

Parameters	Asthma Exacerbation Subjects	Control Subjects	<i>p</i> Value
Age, months	72.06 ± 21.94	82.04 ± 24.19	0.188
Male, n (%)	12 (57.1)	13 (72.2)	0.328
Duration of asthma, years	2.05 ± 1.7	—	
Family history of asthma or atopy, n (%)	14 (66.7)	—	
History of other atopic disease, n (%)	16 (76.2)	—	
ICS treatment, n (%)	9 (42.9)	—	
Daily FP dose (mean, µg/day)	250 ± 88.39	—	
Exacerbation severity			
Moderate, n (%)	7 (33.3)		
Severe, n (%)	14 (66.7)		
Hospital days	6.3 ± 1.8		
Prednisone	21 (100%)	—	
Allergen (house dust mite)	21 (100%)	NA	
Eosinophil count, × 10 ⁹ /L	228.57 ± 235.97	NA	
CD19 ⁺ CD23 ⁺ , %	10.57 ± 3.52	NA	
Total IgE, IU/L	579.24 ± 713.05	NA	
FEV ₁ , % predicted	77.14 ± 8.16	NA	
FeNO, ppb	18.48 ± 10.07	NA	

Data are presented as mean ± SD. ICS = inhaled corticosteroid; FP = fluticasone propionate; CD = cluster of differentiation; IgE = immunoglobulin E; FEV₁ = forced expiratory volume in one second; FeNO = fractional exhaled nitric oxide; NA = not available.

technique. IFN-γ, IL-4, and IL-10 analyses were performed with ELISA kits (B&D, Shanghai, China). sB7-H3 analyses ELISA kits were produced by our laboratory as previously described.¹⁸

Data Analysis

Mean and standard deviation were calculated for the continuous variables regarding to level of cytokines and sB7-H3. The Student's *t*-test was employed for the comparisons. Pearson's correlation test or partial correlation was used to assess the correlations between sB7-H3, level of cytokines, FEV₁ and fractional exhaled nitric oxide based on the normal distribution of all data. A two-sided value of *p* < 0.05 was considered statistically significant. All of the analyses were performed using the Statistical Package for SPSS for windows, version 17.0.

RESULTS

Demographic Data and Clinical Characteristics of Asthma Exacerbation and Control Subjects

Twenty-one children with allergic asthma exacerbation and 18 control children with selective surgery were enrolled in this study. The demographic data and clinical characteristics of the study groups are presented in Table 1. There was no statistical significance in age and gender between asthma exacerbation and

control subjects. All asthma exacerbation subjects had house dust mite sensitization.

B7-H3 Molecule Expression on PBMCs in Asthma Children

The expression of B7-H3 molecule on PBMCs in asthmatic and healthy children was determined by using flow cytometry assay. As shown in Fig. 1, high expression of B7-H3 was found on CD14⁺ monocytes in children with asthma exacerbation compared with healthy controls. No difference of B7-H3 expression on other PBMCs was found.

Soluble B7-H3 and Cytokines Levels of Asthma Exacerbation and Control Subjects

As shown in Fig. 2, on admission, sB7-H3 in plasma were detectable in all the subjects, but those with asthma exacerbation had significantly higher levels of sB7-H3 than control subjects (4.46 ± 1.33 versus 3.42 ± 1.48 ng/mL; *p* = 0.027). Plasma IL-4 level was significantly higher in asthma exacerbation subjects than control subjects (157.98 ± 21.57 versus 121.92 ± 24.37 pg/mL; *p* < 0.0001) as well as IFN-γ level was significantly lower in asthma exacerbation subjects than control subjects (292.73 ± 152.47 versus 421.78 ± 145.84 pg/mL; *p* = 0.0107). No significance was found in level

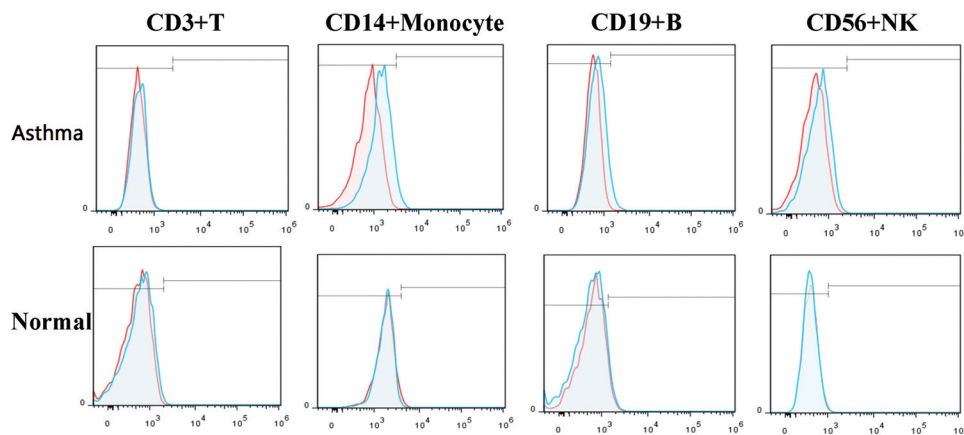


Figure 1. B7-H3 molecule expression on PBMCs in asthma exacerbation and healthy children. The blue lines indicate the staining with anti-B7-H3 mAb, and the red lines indicate background staining with control IgG. High expression of B7-H3 was shown on the membrane of monocyte.

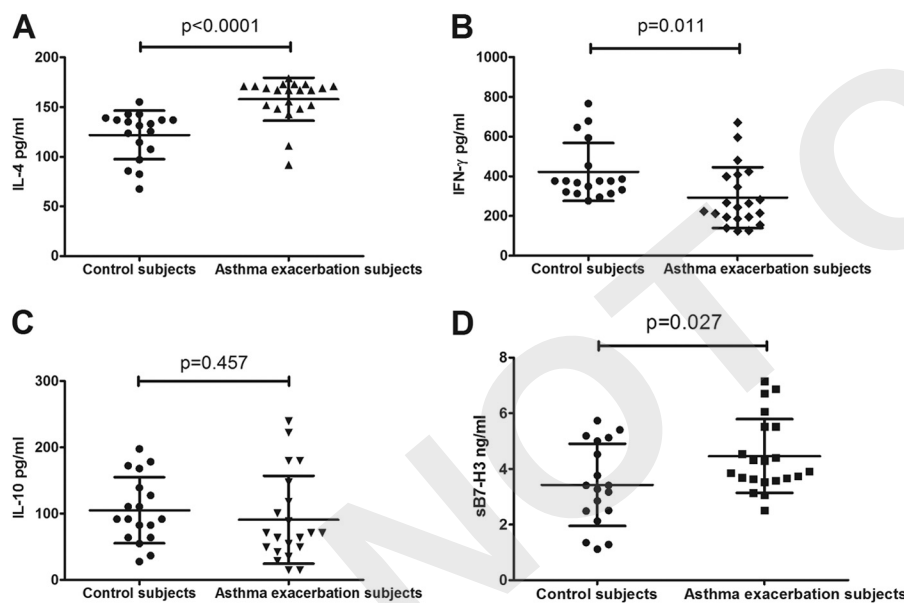


Figure 2. Comparisons of sB7-H3 (A), IL-4 (B), IFN- γ (C), and IL-10 (D) levels in serum between acute asthma exacerbation subjects and control subjects.

of IL-10 between two groups (90.86 ± 66.16 versus 105.16 ± 49.85 pg/mL; $p = 0.457$).

Interestingly, sB7-H3 level of asthma exacerbation subjects with inhaled corticosteroid (ICS) treatment recently was significantly lower than subjects without inhaled corticosteroid (ICS) treatment ($t = 2.706$; $p = 0.0136$), as shown in Fig. 3.

Correlation Between sB7-H3 and IFN- γ , IL-4, IL-10, and Other Clinical Characteristics of Asthma Exacerbation Subjects on Admission and Discharge

Level of sB7-H3 in asthma exacerbation subjects was inversely correlated with level of IFN- γ ($r_p = -0.644$; $p = 0.002$) in plasma and inversely correlated with FEV₁ ($r_p = -0.499$; $p = 0.0212$) on admission. After adjustment with partial correlation, only level of IFN- γ was correlated with sB7-H3 ($r_p = -0.605$; $p = 0.005$) as shown in Fig. 4. No statistical significance was found between sB7-H3 and other cytokines or clinical characteristics both on admission and discharge (Table 2).

Soluble B7-H3 Levels in Plasma Before and After Prednisone Treatment

Ten subjects were randomly detected sB7-H3 of paired plasma on discharge, and levels of sB7-H3 were decreased significantly on convalescent phase in all the observed cases that compared with the value of acute phase (Fig. 5).

DISCUSSION

Identification of biologic markers from asthma children and development of assays to measure them is an important goal in asthma evaluation. The study of soluble costimulatory molecules in allergic asthma, particularly in children, is presently still very limited and focuses on cytotoxic T lymphocyte-associated antigen-4, sCD28, sCD80, and sCD86.^{23,24} To our knowledge, this is the first investigation of sB7-H3 level in children with allergic asthma exacerbation.

To explore whether level of sB7-H3 is related to asthma exacerbation, we describe a quantitative mea-

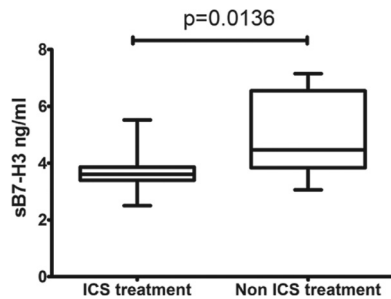


Figure 3. Comparison of sB7-H3 level between acute asthma exacerbation subjects with ICS treatment and non-ICS treatment.

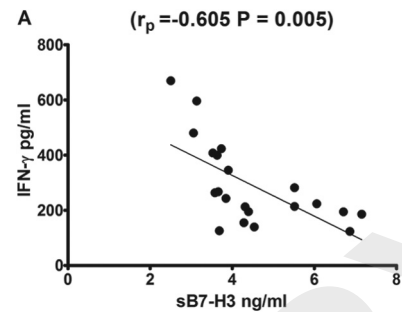


Figure 4. Correlations between sB7-H3 level and IFN- γ .

surement of circulating B7-H3 expression in plasma samples from children with allergic asthma exacerbation. In the present study, compared with the expression of B7-H3 in control children, membrane B7-H3 on CD14⁺ monocytes and circulating B7-H3 level was significantly increased in allergic asthma exacerbation as well as the level of sB7-H3 in asthma exacerbation subjects was inversely correlated with the level of IFN- γ in plasma. These results indicate that sB7-H3 might play a role in asthma exacerbation.

In allergic asthma, allergen-specific T lymphocytes are activated and strongly implicated in the pathogenesis of asthma,^{25,26} with the participation of costimulatory signals such as B7 family molecules. Our previously study²⁷ indicates that the anti-B7-1 antibody treatment could alleviate asthmatic syndromes of asthma model induced by ovalbumin, reduce infiltration of eosinophils, increase the expression level of IFN- γ , and decrease the expression level of IL-4 in bronchoalveolar lavage fluid. Blocking mAb to B7-2 also inhibits pulmonary eosinophilia infiltration, specific immunoglobulin E production, and AHR in mice.¹² Recently, a study showed that administration

of anti-B7-H3 mAb significantly reduced AHR with a concomitant decrease in eosinophils in the lung and decreased production of Th2 cytokines (IL-4, IL-5, and IL-13) in the draining lymph node cells in asthma model,²⁸ which also indicated that B7-H3 play an important role in the asthmatic responses through facilitating the priming of pathogenic Th2 cells; however, there are limited human studies about the association of B7-H3 and asthma. In the present study, the plasma level of IL-4 was increased and IFN- γ level was decreased in asthma exacerbation as same as other studies,^{29,30} and the level of sB7-H3 in asthma exacerbation subjects was inversely correlated with the level of IFN- γ , but no correlation was found between sB7-H3 and IL-4. These indicate that B7-H3 may have inhibitory functions, especially for Th1 cells, in certain circumstances like asthma exacerbation. B7-H3-deficient mice developed more severe airway inflammation than did wild-type mice in conditions in which Th cells differentiated toward type 1 as well as B7-H3-deficient mice developed experimental autoimmune encephalomyelitis several days earlier than their wild-type littermates.¹⁴ These results suggested that B7-H3 is a nega-

Table 2 Correlation between sB7-H3 and various cytokines and clinical characteristics of asthma exacerbation subjects

Clinical Parameters	sB7-H3 on Admission		sB7-H3 on Discharge	
	21 Subjects		10 Subjects	
	r_p	p Value	r_p	p Value
IFN- γ (pg/mL)	-0.644**	0.002	-0.340	0.337
IL-4 (pg/mL)	0.256	0.264	0.239	0.506
IL-10 (pg/mL)	-0.075	0.748	0.140	0.700
FEV ₁ (% predicted)	-0.499*	0.0212	-0.313	0.378
FeNO (ppb)	0.084	0.717	0.276	0.440
Eosinophil count (/mm ³)	0.062	0.790	0.370	0.293
Total IgE (IU/mL)	-0.139	0.547	-0.319	0.369
CD19 ⁺ CD ⁺ 23 (%)	-0.073	0.752	0.221	0.540
Hospital days	-0.062	0.789	-0.301	0.397

* $p < 0.05$; ** $p < 0.01$. IFN- γ = interferon- γ ; IL = interleukin; FEV₁ = forced expiratory volume in one second; FeNO = fractional exhaled nitric oxide; CD = cluster of differentiation; IgE = immunoglobulin E.

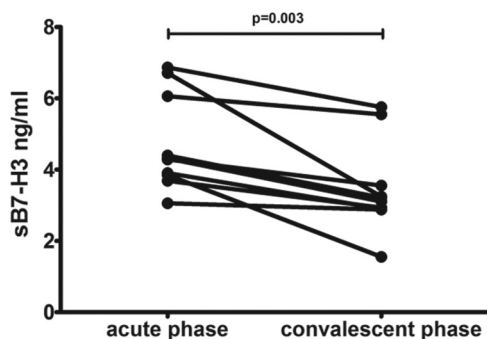


Figure 5. Soluble B7-H3 levels in serum before and after treatment in asthma exacerbation subjects.

tive regulator that preferentially affects Th1 responses. However, several former studies demonstrated that B7-H3 as a positive costimulatory molecule for IFN- γ induction in activated human T cells.^{13,31} Recently, our research team demonstrated that h2IgB7-H3 enhanced T-cell proliferation and increased IFN- γ and IL-2 releasing, whereas h4IgB7-H3 down-regulated the activation of T cells. These results indicated two receptors for B7-H3 with opposing functions might exist on activated T cells through three dimensional reconstruction structure models of two isoforms and fusion-protein binding assays.³² Further studies are needed to perform to prove the two putative receptors.

Recent studies have reported IL-10 secreted by regulatory Th cells able to suppress human allergen-driven Th2 cytokine production of IL-4-producing effector cells.⁸ forkhead box P3, the regulatory T-cell transcription factor, controlled Th2 inflammation by regulating innate immune cell recruitment through an IL-10-dependent pathway and the protective effects of forkhead box P3 could be reversed by depletion of IL-10.³³ Their studies implied that IL-10 participate the immunopathogenesis of asthma. However, in the present study, level of plasma IL-10 in asthma exacerbation subjects had lower levels than controls; however, the difference was not statistically significant. This result is similar to the study performed by Tsai *et al.*,³⁴ and no correlation was found between level of sB7-H3 and IL-10.

In present study, we found subjects with ICS treatment had lower level of circulating B7-H3 than subjects without ICS treatment. Meanwhile, levels of sB7-H3 were decreased significantly on convalescent phase than acute phase after prednisone medication. These results show that glucocorticoid may play a part in asthma treatment through inhibiting expression of sB7-H3. Thus, level of sB7-H3 maybe a good parameter for evaluation of asthma severity.

CONCLUSIONS

Costimulatory molecule B7-H3 may play an important role in asthma exacerbation in children without

ICS treatment, especially for decreasing IFN- γ level, which results in Th2 bias to some extent. Studies are in progress to determine the precise pathophysiological role of B7-H3 during asthma exacerbation. However, some limitations of this study should be noted. Firstly, the present study was based on a single center for data and just included 21 patients, which might have potential biases. Secondly, this study just showed the associations between sB7-H3 and clinical profiles rather than the direct proofs between sB7-H3 and asthma. In addition, there was no follow-up information of asthma patients who had different levels of sB7-H3. Thus, further studies in large, well-characterized patient samples are needed to confirm and explore the clinical applications of these observations.

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