

T_H17 Cell Frequency in Peripheral Blood from Children with Allergic Asthma Correlates with the Level of Asthma Control

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Here we show that the frequency of T_H17 cells (CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes) is increased in peripheral blood of children with allergic asthma. Moreover, we found a significant relationship between the frequency of T_H17 cells and level of asthma control, with reduced asthma control correlated with a higher proportion of T_H17 cells. (*J Pediatr* 2012; ■: ■-■).

In Western countries, asthma is the most frequent chronic disease in childhood.¹ For many years, the paradigm of underlying immune dysregulation dominated mainly by T helper (T_H) 2 cells was widely accepted. With the recent description of T_H17 cells and further T_H cell subtypes, new aspects have been added to the understanding of immune regulation during the allergic response.²

Although much of the data on the role of T_H17 immunity in the allergic response are derived from animal models,³ it has been shown that interleukin (IL)-17 is increased in sputum and bronchoalveolar lavage fluid from patients with asthma.⁴ Subsequent studies implied a role for IL-17 in perpetuating allergic inflammation in asthma by enhancing the local accumulation of neutrophils.⁵ A recent study reported an increased T_H17 response in adults with allergic asthma.⁶ The only previous study addressing the role of T_H17 in pediatric allergy found that enhanced allergen-induced IL-17 responses during immunotherapy were associated with high clinical symptom scores in children with allergic rhinitis.⁷ However, although childhood represents the most critical period for the development of asthma, T_H17 immunity has not yet been studied in this context. Thus, the aim of the present study was to evaluate whether the frequency of T_H17 cells (CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes) is increased in children with allergic asthma and is correlated with disease activity.

Methods

Twenty-five children with allergic asthma were diagnosed according to standardized criteria.⁸ Children with nonallergic asthma were excluded. Asthma control was classified as controlled, partly controlled, or uncontrolled, according to Global Initiative Against Asthma guidelines.⁹ The use of inhaled steroids did not differ significantly among the 3 asthma groups (χ^2 test; $P = .46$). Patient characteristics are summarized in the [Table](#).

A control group of 15 healthy children was recruited. Exclusion criteria for the control group were symptoms or a doctor's diagnosis of pulmonary or allergic disease, chronic immune disorder, acute infection (assessed clinically and by laboratory measurements) during the previous 2 weeks, and 1 or more positive items on the modified asthma predictive index.¹⁰ Children with sensitization detected by a radioallergen sorbent test or skin prick test were not included in the control group.

After informed consent, 1.2 mL of venous blood was collected during a routinely performed blood withdrawal. The study was approved by the local Ethics Committee. Erythrocytes were lysed, and peripheral blood mononuclear cells were recovered by density centrifugation using a Ficoll-Hypaque gradient (PAA, Linz, Austria).

After washing, cells were stained with the following fluorescence-labeled antibodies: anti-CD3 (FITC), anti-CD4 (PerCP), anti-CD161 (PE) (all from BD Biosciences, Heidelberg, Germany), and anti-CCR6 (AlexaFluor 647; Biozol, Eching, Germany). Isotype controls were purchased from BD Biosciences. Analyses were performed with a 4-color FACS Calibur flow cytometer (BD Biosciences). Cells were gated into a physical lymphocyte gate in the forward scatter/side scatter plot. T_H17 cells were defined as CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes.¹¹ At least 30 000 lymphocytes were analyzed in each sample.

Statistical analyses were performed using Prism 5.0 (GraphPad Software, La Jolla, California) and SPSS version 17.0 (SPSS Inc, Chicago, Illinois). Normal distribution was assessed using the Kolmogorov-Smirnov test. Differences were assessed with the 2-tailed Student *t* test for normally distributed data and the Mann-Whitney *U* test for nonnormally distributed data. The level of statistical correlation was determined by Pearson product-moment correlation. Adjustment

T _H	T helper
IL	Interleukin

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Table. Patient characteristics

	No allergies or asthma	Allergic asthma		
		Controlled	Partly controlled	Uncontrolled
Number of patients	15	14	8	3
Age, years, mean $\pm$ SEM (range)	5.2 $\pm$ 1.2 (1-16)	9.5 $\pm$ 1.2 (2-17)	11.8 $\pm$ 1.8 (4-17)	9.7 $\pm$ 1.2 (8-12)
Sex, males/females, n	6/9	9/5	5/3	3/0
Inhaled steroids on presentation, n	0	4	3	2
Eosinophils, %	3.1 $\pm$ 0.5	5.7 $\pm$ 1.0	10.1 $\pm$ 4.3	6.0 $\pm$ 3.5
Eosinophils, 1000/ μ L	0.2 $\pm$ 0.1	0.4 $\pm$ 0.1	1.1 $\pm$ 0.6	0.4 $\pm$ 0.2
Neutrophils, %	42 $\pm$ 2.5	51 $\pm$ 2.4	44 $\pm$ 4.4	48 $\pm$ 5.6
Neutrophils, 1000/ μ L	3.2 $\pm$ 0.5	3.9 $\pm$ 0.4	3.1 $\pm$ 0.6	3.7 $\pm$ 0.6
Total serum IgE, kU/L	23 $\pm$ 7	416 $\pm$ 104	812 $\pm$ 419	915 $\pm$ 421
FEV ₁ , % predicted		103 $\pm$ 3	88 $\pm$ 6	83 $\pm$ 10
MEF ₇₅₋₂₅ , % predicted	NA	95 $\pm$ 5	67 $\pm$ 9	55 $\pm$ 2

FEV₁, forced expiratory volume in 1 second; MEF, maximal expiratory flow; NA, not applicable.

for age was done using a linear regression model. Data are reported as mean $\pm$ SEM.

Results

In healthy children, T_H17 cells (CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes) accounted for 2.9% $\pm$ 0.5% of all T_H cells (Figure); this proportion was significantly increased to 6.9% $\pm$ 0.7% in children with allergic asthma ($P < .001$).

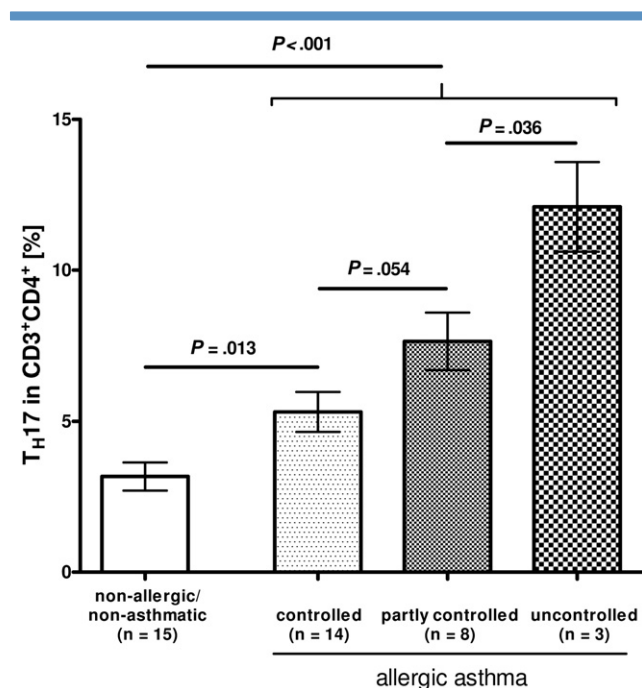


Figure. Compared with healthy, non-allergic/non-asthmatic children, frequency of T_H17 cells (CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes) was increased in children with allergic asthma ($P < .001$). Subanalysis revealed a correlation between T_H17 frequency and level of asthma control, with reduced asthma control was correlated with increased proportion of T_H17 cells. Data are mean $\pm$ SEM.

Absolute T_H17 numbers reached 0.037 $\pm$ 0.004 G/L in healthy controls and were doubled in children with asthma (0.077 $\pm$ 0.010 G/L; $P = .002$) (data not shown). Correlation analysis revealed no significant age dependency in either group ($R^2 = 0.12$, $P = .21$ for healthy controls and $R^2 = 0.06$, $P = .25$ for children with asthma). After adjustment for age, the P value for the difference in T_H17 frequency between the 2 groups was .013.

Further analysis with subclassification of the group with asthma according to levels of asthma control revealed a correlation between frequency of T_H17 cells and level of asthma control. In children with controlled asthma, CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes accounted for 5.3% $\pm$ 0.7% of all T_H cells ($P = .013$ compared with healthy children). This proportion was increased to 7.6% $\pm$ 0.9% in children with partly controlled asthma ($P = .054$ compared with children with controlled asthma) and markedly increased to 12.1% $\pm$ 1.5% in the children with uncontrolled allergic asthma ($P = .036$ compared with children with partly controlled asthma). However, the number of children with uncontrolled asthma was low. Compared with the healthy control group, the increase in T_H17 frequency was significant for all 3 subgroups ($P = .013$ for children with controlled asthma; $P < .001$ for children with partly controlled and uncontrolled asthma).

Discussion

In this study of the role of T_H17 cells in childhood asthma, we detected a significant increase in the frequency of T_H17 cells (defined as CD3⁺CD4⁺CD161⁺CCR6⁺ lymphocytes¹¹) in the peripheral blood of children with allergic asthma compared with healthy controls. We found a relationship between the frequency of T_H17 cells and level of asthma control, with reduced asthma control correlated with a higher proportion of T_H17 cells. Our findings are analogous to those reported by Ciprandi et al,¹² which showed an association between serum IL-17 level and clinical disease activity in allergic rhinitis. Thus, it might be interesting in a future study to correlate peripheral T_H17 cell frequency with concentrations of IL-17 in plasma and other

compartments (eg, sputum). McKinley et al¹³ reported that T_H17 cells mediated steroid-resistant neutrophilic airway inflammation in a murine model. Among the 6 members of the IL-17-family, IL-17E increases IL-4/IL-13 (T_H2) inflammation, and IL-17A and IL-17F induce neutrophilic (T_H1) inflammation via IL-6/IL-8.⁶ Because patients with severe asthma display increased numbers of neutrophils in the airways,¹⁴ IL-17A- or IL-17F-secreting T_H17 cells might contribute to their recruitment.^{2,3,15} Thus, it would be informative to characterize the T_H17 response in children with steroid-resistant asthma in a future study.

In conclusion, our data indicate an increased frequency of peripheral blood T_H17 cells in children with allergic asthma, and a correlation with the level of asthma control. ■

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