Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Passive smoking induces pediatric asthma by affecting the balance of Treg/Th17 cells

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BACKGROUND: We aimed to explore the effects of passive smoking on the severity of pediatric asthma and associated molecular mechanisms.

METHODS: A total of 378 children with asthma were assigned into four groups according to asthma severity (from grades I to IV). Univariate and multivariate regression analyses were used to analyze possible factors associated with asthma severity in children. Environmental tobacco smoke (ETS) exposure was measured via cotinine concentration in urine. Serum levels of immunoglobulin E (IgE) and cytokines were measured using allergen diagnostic and ELISA (enzyme-linked immunosorbent assay) kits. The percentage of T-regulatory (Treg) and T-helper type 17 (Th17) cells in peripheral blood mononuclear cells (PMBCs) were measured by flow cytometry. Treg- and Th17-associated transcription factors from PMBCs were measured by using ELISA kits.

RESULTS: The levels of ETS and serum IgE, and the duration and amounts of passive smoking were closely associated with asthma severity. Passive smoking significantly reduced the levels of FoxP3 (Forkhead/winged helix transcription factor) and tumor growth factor-β, which were associated with Treg cells, and increased the levels of interleukin-17A and interleukin-23, which were associated with Th17 cells. Meanwhile, passive smoking significantly reduced the ratio of Treg/Th17 cells ($P < 0.05$).

CONCLUSIONS: Passive smoking was closely associated with the severity of childhood asthma by affecting the balance of Treg/Th17 cells.

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INTRODUCTION

Asthma is a major chronic disease that seriously affects child health worldwide.[1] In recent years, the morbidity and mortality caused by asthma is increasing.[2] Cigarette smoke is an important factor inducing asthma.[3] Children are exposed to cigarette smoke and will be affected by passive smoking, so understanding the relationship between passive smoking and asthma in children is of great significance in the prevention of childhood asthma. Smoking has significant negative effects on the respiratory system of asthmatic patients, not only causing frequent asthma and destroying lung function of patients, but also reducing the therapeutic results of oral glucocorticoids.[4]

Nicotine, one of the major components of cigarette smoke, has immunomodulatory effects that can affect immune surveillance and promote disease development. There is consistent evidence for that nicotine is an immunomodulatory agent that has unique effects on host immunity.[5] Passive smoking makes the asthma complex and pathophysiological processes complicated, and its clinical features also vary. The impact of passive smoking on airway inflammation and remodeling of asthma remains unclear. Effects of short-term smoking on respiratory inflammation have been proved in ovalbumin (OVA)-stimulated mice.[6]

The imbalance of T-helper (Th) cell subpopulations is an important factor in the pathogenesis of asthma. Therefore, the effects of the balance of regulatory T cell (Treg)/Th17 cell on the pathogenesis of asthma have been of great interest to researchers.[7] It is speculated that smoking may affect the balance of Treg/Th17 cells in asthma and thus aggravates airway inflammation and remodeling of asthma. Treg cell, a type of $CD4^+$ T cell, is an important subset that negatively regulates the immune response of foreign antigens. It can secrete cytokines such as interleukin-10 (IL-10) and tumor growth factor-β (TGF-β), as well as against foreign pathogens.[8] Treg cell plays an important role in the prevention of asthma.[9] $CD4^+CD25^+$ Treg is a major component of Treg cell, which is from the thymus and exists in peripheral immune organs, accounting for 5–10% of $CD4^+$ T cells in the peripheral blood. Forkhead/winged helix transcription factor (Foxp3) is highly expressed in $CD4^+CD25^+$ T cells.[10]

The effect of passive smoking on the percent of Treg cells and Foxp3 expression remains unclear. Transcription factors regulate the production of cytokines and inflammatory mediators, and become a hot research topic in inflammatory diseases. Transcription factors are expected to become a new therapeutic target.[11] In this study, the impact of passive smoking on immune balance of Treg/Th17 cells was explored. It is generally accepted that smoking has an important impact on the occurrence and development of asthma. However, there are few reports about the molecular mechanism between the clinical manifestations of asthma and passive smoking in children. This study focused on the effects of passive smoking on childhood asthma.

MATERIALS AND METHODS

Participants

All procedures were approved by the Ethical Human Research Committee of Affiliated Hospital of Changchun University of

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Traditional Chinese (IRB number: CN-2016-0061). A total of 378 children diagnosed with asthma were recruited at Affiliated Hospital of Changchun University of Traditional Chinese from March 2016 to May 2017.

The diagnosis of asthma was established according to National Heart, Lung, and Blood Institute (NHLBI) guidelines,[12] and severe asthma was defined using European Respiratory Society/American Thoracic Society guidelines.[13] All asthmatic children had reversible airflow obstruction after an inhaled short-acting β_2-agonist or airway hyper-reactivity in methacholine bronchoprovocation testing.

A unified epidemiological questionnaire was used to investigate the basic situation of children, including parents smoking, living habits, and passive smoking of children. The degree of passive smoking was classified as environmental tobacco smoke (ETS) according to an earlier report.[14] ETS0, no smoking in room; ETS1, ≤9 cigarettes smoked daily in room; ETS2, ≤10 and <20 cigarettes smoked daily in room; ETS3, ≥20 cigarettes smoked daily in room.

Recurrent asthma, wheezing, short breath, and cough are often associated with passive smoking. Diffuse sound can be heard in the lungs, and exhaled wheeze and expiratory phase will be prolonged. All the patients received bronchodilator test, serum allergen examination, sputum-induced eosinophil count, and fractional concentration of exhaled nitric oxide (FeNO) measurement. Pulmonary function was tested by using the pulmonary function instrument (model number: HI801, JECT, Japan). FeNO value was determined by using a NO detector (Sunvou, SU-02, Wuxi Shangwo Biological Technology Co., Ltd., Wuxi, China); FeNO (ppb) (1ppb = 1×10 mol/l). Bronchial provocation test or positive bronchodilator test was also performed. The following standard for asthma was used: positive bronchodilator test (forced expiratory volume in one second (FEV_1) >12% or more, absolute FEV_1 increased ≥200 ml; maximum expiratory flow (PEF) intraday variability or diurnal fluctuation rate ≥20%). Grades of asthma severity were classified by using the contents from Table 1.

Measurement of ETS exposure

Air pollution in Changchun City was downloaded from local weather broadcast service as Table 2 showed. Most days of Air Quality Indexes (AQI) were good, 0–50 or moderate, 51–100. Urine was collected from all subjects on the morning of the day of investigation in June 2017. ETS exposure was measured according to patients' urine cotinine levels, and the cut-off was 30 ng/mg.[15] Urine cotinine levels were measured by using a Cotinine Direct ELISA kit from Calbiotech Inc. (El Cajon, CA, USA).

Serum IgE measurement

Total immunoglobulin E (IgE) and airway hyper-responsiveness were associated with childhood asthma.[16] Serum levels of IgE were measured by using Allergen diagnostic kit (Cat. No.: K-m120713, GmbH Medical Company, Karlsruhe, Germany).

Inclusion criteria

The following inclusion criteria were used for all the children: their parents and or other family members were smokers; the children lived with the smokers who were the caregivers of children; all children were from the same city, Changchun; their ages were from 4 to 12 years old; their caregivers were willing to cooperate and submitted signed informed consents; the child often sneezed, although they had no a cold; purple skin was caused by allergy; there was obvious paroxysmal chest tightness and wheezing cough; elevated serum allergen-specific IgE concentrations were more than 150 IU/ml.[17] Meanwhile, some children living with non-smokers were also selected as controls.

Exclusion criteria

The following exclusion criteria were used for the children: their parents or grandparents had a history of asthma; the family smokers did not live with the children; parents had a history of passive smoking; children had serious illnesses such as heart disease and mental disorders; medication was used within 4 weeks; the children had respiratory or bacterial infections or infectious diseases in the previous month; the patients had allergic rhinitis within 1 month; and their parents were drinkers.

Specimen collection

Three milliliter of blood was obtained from each patient by using a tube with heparin anticoagulant. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood of by Ficoll-Hypaque (Solarbio Chemical Company, Beijing, China) density centrifugation. Two milliliters of peripheral blood was placed in a non-anticoagulant tube and stored at 25 °C for 1 h and centrifuged at $2000 \times g$ for 5 min at 25 °C. The serum was prepared and stored at −80 °C in a freezer.

Measurement of Treg and Th17 cell molecules

Serum TGF-β, IL-17A and IL-23, and FoxP3 from PBMCs were measured by human kits. Human TGF-β,FoxP3, IL-17A, and IL-23 ELISA kits were purchased from Jingke Hongda Biotechnology Company (Beijing, China).

The contents of Treg cells and Th17 cells in the $CD4^+$ cell population

RPMI-1640 medium, phorbol myristate acetate (PMA), ionomycin, and monensin were purchased from Beijing Biotechnology Institute of China (Beijing, China). CD25-PE-A and FoxP3 PerCP-Cy5.5 antibodies were purchased from eBioscience (Cat.No.45-4776-42, CA). PBMCs were adjusted to 1×10^6/ml. Five hundred microliters of PBMCs were added to 500 µl RPMI-1640 medium with 20 ng/ml PMA, 1 pg/ml ionomycin, 500 ng/ml monenomycin, and cultured at 37 °C in a 5% CO_2 incubator for 4 h, and washed twice with phosphate-buffered saline (PBS). $CD4^+$ cells were gated from the lymphocyte population in forward scatter/side scatter. The percentage of Treg and Th17 cells in $CD4^+$ T cell population was analyzed by a flow cytometer (Beckman Coulter, CA, USA). Twenty microliters of CD25-PE-A and PerCP-Cy5.5 antibodies were added to the tubes and incubated for 30 min. The mixture was washed by PBS for one time and centrifuged at $350 \times g$ for 5 min, and the supernatant was discarded. One milliliter of fixative was added and mixed well, and kept at 4 °C for 12 min. Two millilters of permeate was added and centrifuged at $350 \times g$ for 5 min, and the supernatant was discarded. Twenty microliters of Foxp3-PE was added to the experimental tube and 20µl of IgG2a-PE was added to a control tube. The mixture was incubated at 4 °C for 30 min. Two milliliters of permeate was added and centrifuged at $350 \times g$ for 5 min, and the supernatant was discarded. PBS (0.5 ml) was added and the levels of $CD4^+CD25^+$ Treg cells were analyzed by flow cytometry. The percentage of Th17 cells was measured by using flow cytometry and the Human Th17 Flow kit (BioLegend, San Diego, CA, USA).

Statistical analysis

With clinical results, the possible factors affecting asthma were selected and quantified. Univariate analyses were performed among different groups in asthma severity from grades I to IV. χ^2 test, analysis of variance, univariate logistic, and multivariate regression analyses were performed to analyze the association between asthma and one of the following dependent outcome variables, including age, sex distribution, body mass index (BMI), birth time, birth weight, passive smoking, serum IgE, FEV_1/forced vital capacity (FVC), FEV_1 improvement, FeNO, and or induced sputum eosinophil count. Statistical analysis was carried out by using SPSS soft package 20.0 (SPSS Inc., IBM, NY, USA).

Table 1. The grades of asthma severity (44)

Asthma grades	Lung function	Clinical characteristics
Grade I, intermittent	FEV_1≥80% predicted value or PEF ≥80% personal best value, PEF or FEV_1 decline rate <20%	Symptoms occurring <1 time per week, transient, night symptoms ≤2 times per month
Grade II, mild persistent	FEV_1≥80% predicted value or PEF ≥80% personal best value, PEF or FEV_1 decline rate of 20–30%	Symptoms occurring ≥1 time per week, but <1 time daily, may affect daily life and sleeping, night asthma >2 time per month, but <1 time per week
Grade III, moderate persistent	FEV_1 60–79% predicted value, PEF 60–9% personal best values, PEF or FEV_1 decline rate >30%	Symptoms occurring daily and affect daily life and sleeping, night asthma ≥1 time per week
Grade IV, severe persistent	FEV_1<60% predicted values, PEF <60% personal best value, PEF or FEV_1 decline rate >30%	Frequent daily and night symptoms, significantly affect physical activity

FEV_1 forced expiratory volume in one second, *PEF* maximum expiratory flow

Table 2. Air pollution in Changchun City in June 2017

Date	AQI	PM2.5	PM10	SO_2 (μg/m^3)	NO_2 (μg/m^3)	CO (mg/m^3)	O_3 (μg/m^3)
2017-6-01	45	18	49	10	34	0.86	45
2017-6-02	31	12	29	10	31	0.84	41
2017-6-03	44	20	40	11	40	0.94	47
2017-6-04	36	12	28	11	41	0.86	54
2017-6-05	57	17	46	9	43	0.9	71
2017-6-06	57	20	56	11	54	1.01	77
2017-6-07	49	30	45	10	33	1.07	80
2017-6-08	46	17	41	10	34	0.94	69
2017-6-09	56	14	64	11	37	0.86	41
2017-6-10	38	13	40	12	32	0.81	30
2017-6-11	46	18	51	12	34	0.9	32
2017-6-12	52	21	58	14	44	0.95	45
2017-6-13	79	29	86	12	50	1.05	76
2017-6-14	70	26	72	14	46	1.04	99
2017-6-15	53	17	55	12	43	0.87	64
2017-6-16	73	15	49	11	44	0.85	101
2017-6-17	91	44	95	13	53	1.31	138
2017-6-18	107	39	79	11	26	1.12	157
2017-6-19	81	30	51	9	27	1.09	132
2017-6-20	60	18	29	9	27	0.97	105
2017-6-21	41	14	26	9	30	0.89	74
2017-6-22	44	14	38	9	36	0.93	68
2017-6-23	70	24	71	9	60	1.11	72
2017-6-24	72	30	84	10	70	1.22	60
2017-6-25	52	27	54	8	55	1.01	52
2017-6-26	47	14	37	8	38	0.84	63
2017-6-27	77	24	58	9	47	1.04	101
2017-6-28	74	24	56	9	37	0.94	115
2017-6-29	73	26	63	9	25	0.97	125
2017-6-30	45	53	85	9	31	1.26	124

AQI air quality index, *PM* particulate matter

RESULTS

Demographic characteristics

Lung function FEV_1/FVC, FEV_1 improvement, FeNO, and induced sputum eosinophil count were increased with the increase in asthma grades (Table 3). The statistical differences were significant for pulmonary function (FEV_1/FVC), inhalation bronchodilator FEV_1 improvement rate, FeNO, and sputum eosinophil count among different groups with different severities of asthma ($P < 0.05$, Table 3). The statistical differences were also significant for ETS grades and the duration of passive

Table 3. Demographic characteristics

Parameters	Asthma grade I	Asthma grade II	Asthma grade III	Asthma grade IV	χ^2 and t value	P value
Cases	103	158	86	31		
Gender, male/female	62/41	97/61	49/37	17/14	0.762	0.858
Age (years)	8.38 ± 4.11	8.26 ± 4.15	8.24 ± 4.27	8.13 ± 3.76	0.207	0.465
The time of birth, cases (%)						
Pre-term	14	20	9	3	1.1362	0.979
Full term	82	129	73	26		
Post term	7	9	4	2		
Birth weight, cases (%)						
Low	8	11	7	3	1.624	0.951
Normal	76	119	65	25		
High	19	28	14	3		
BMI	23.47 ± 3.06	23.14 ± 2.91	22.48 ± 2.75	23.08 ± 3.24	0.568	0.179
Prenatal smoking, cases (%)	8 (7.77)	13 (8.23)	11 (12.79)	6 (19.35)	4.86	0.18
ETS classification						
ETS0	75	11	5	1	269.013	0.000
ETS1	18	102	26	7		
ETS2	9	38	37	7		
ETS3	1	7	14	16		
Passive smoking duration, cases (%)						
0–3 years	94	27	11	3	151.398	0.000
4–6 years	6	83	16	5		
7–9 years	2	36	39	6		
10–12 years	1	12	16	17		
FEV_1/FVC (%)	70.46 ± 6.51	62. 41 ± 6. 02	55. 43 ± 5. 83	45.20 ± 6.72	47.754	0.000
FEV_1 improvement (%)	3.26 ± 1.37	12.53 ± 2.78	17. 09 ± 3. 17	37. 75 ± 4.38	276.385	0.000
FeNO (ppb)	16.38 ± 2.58	21.46 ± 2.88	30.25 ± 3.51	39.20 ± 6.23	98.721	0.000
Induced sputum eosinophil count (%)	5. 03 ± 1.46	20.41 ± 5.27	26.70 ± 14.73	30.76 ± 5.39	179.584	0.000

Note: BMI, weight (kg)/height(m)^2. ETS was classified as according to the degree of passive smoking. Pre-term, 35.8 (32.4–37.9) weeks; full term, 40.1 (38.0–42.0) weeks; and post term 42.4 (42.1–43.0) weeks. ETSO, no smoking in room; ETS1, ≤9 cigarettes smoked daily in room; ETS2, ≤10 and <20 cigarettes smoked daily in room; ETS3, ≥20 cigarettes smoked daily in room. The statistical difference was significant if $P < 0.05$
BMI body mass index, *ETS* environmental tobacco smoke, *FEV_1* forced expiratory volume in one second, *FVC* forced vital capacity, *FeNO* fractional concentration of exhaled nitric oxide

smoking ($P < 0.05$, Table 3). The statistical differences for other parameters were insignificant among different groups ($P > 0.05$, Table 3). The results showed that all the parameters were insignificant for demographic characteristics between the subjects living with non-smoker and smoker groups ($P > 0.05$, Table 4).

The levels of ETS exposure

Urine levels of cotinine were increased significantly from asthma grade I to IV (Fig. 1, $P < 0.05$). Passive smoking aggravated the asthma by increasing the levels of ETS exposure.

Serum levels of IgE

To confirm the changes for the serum levels of IgE, the levels were measured in the patients with different severities of asthma. Serum levels of IgE were increased significantly from asthma grades I to IV (Fig. 2, $P < 0.05$). Passive smoking aggravated the asthma by increasing serum levels of IgE.

The factors associated with the incidence of asthma

Univariate regression analysis showed that the possible factors affecting asthma included age, the amounts and duration of passive smoking, serum IgE levels, FEV_1/FVC, FEV_1 improvement, FeNO, and induced sputum eosinophil count ($P < 0.05$, Table 5). The significant parameters were further analyzed by using multivariate regression. The results showed that the possible factors affecting asthma included the amounts and duration of passive smoking, serum IgE, FEV_1/FVC, FEV_1, FeNO, and induced sputum eosinophil ($P < 0.05$, Table 6).

The effects of passive smoking on the levels of Th17 cell cytokines, and Treg cell transcription factors

Th17 and Treg cells are the two main subsets of T cells involving in asthma.[18] FoxP3 is a biomarker of Treg cells and participants in their function.[19] TGF-β is associated with Treg cell development, and regulates the percentage of Treg cells.[20] IL-17A is an important pro-inflammatory cytokine and mainly produced by Th17 cells.[21] IL-23 is another important cytokine associated with Th17 cells.[22] The levels of FoxP3 (Fig. 3a) and TGF-β (Fig. 3b) were reduced where the serum levels of IL-17A (Fig. 3c) and IL-23 (Fig. 3d) were increased with the progression of asthma ($P < 0.05$). These results indicated that passive smoking affected the ratio of Th17/Treg cells since these molecules were main biomarkers of the two subsets of T cells. Thus, the effects of passive smoking on the ratio of Treg/Th17 cells in different groups were further investigated.

Table 4. Demographic characteristics between the subjects living with non-smoker and smoker groups

Parameters	No smoking	Smoking	χ^2 and t value	P value
Cases	92	286		
Gender, male/female	52/40	173/113	0.455	0.500
Age (years)	8. 26 ± 3. 95	8. 19 ± 4. 18	0.165	0.579
The time of birth, cases (%)				
Pre-term	12	34	0.096	0.953
Full term	79	231		
Post term	5	17		
Birth weight, cases (%)				
Low	8	21	0.193	0.908
Normal	72	213		
High	15	49		
BMI	23.29 ± 3.02	23.03 ± 2.90	0.168	0.318
Prenatal smoking, cases (%)	9 (9.78)	29 (10.14)	0.010	0.921
Passive smoking duration, cases (%)				
0–3 years	32	103	0.143	0.986
4–6 years	27	83		
7–9 years	21	62		
10–12 years	9	31		
FEV_1/FVC (%)	62.14 ± 6.21	62. 47 ± 6.38	0.045	0.589
FEV_1 improvement (%)	11.36 ± 2.51	11.52 ± 2.93	0.078	0.432
FeNO (ppb)	24.18 ± 3.72	23.91 ± 3.25	0.351	0.138
Induced sputum eosinophil count (%)	16.35 ± 7.28	16.63 ± 8.13	0.125	0.264

Note: BMI, weight (kg)/height(m)^2. ETS was classified as according to the degree of passive smoking. Pre-term, 35.8 (32.4–37.9) weeks; full term, 40.1 (38.0–42.0) weeks; and post term 42.4 (42.1–43.0) weeks. ETSO, no smoking in room; ETS1, ≤9 cigarettes smoked daily in room; ETS2, ≤10 and <20 cigarettes smoked daily in room; ETS3, ≥20 cigarettes smoked daily in room. The statistical difference was significant if $P < 0.05$
BMI body mass index, *ETS* environmental tobacco smoke, *FEV_1* forced expiratory volume in one second, *FVC* forced vital capacity, *FeNO* fractional concentration of exhaled nitric oxide

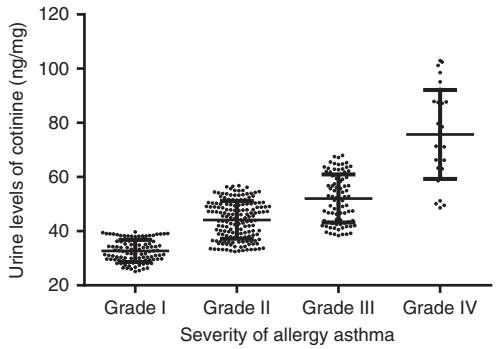

Fig. 1 The levels of environmental tobacco smoke (ETS) exposure in different groups. $n = 103$, 158, 86, and 31 in asthma grades I, II, III, and IV, respectively

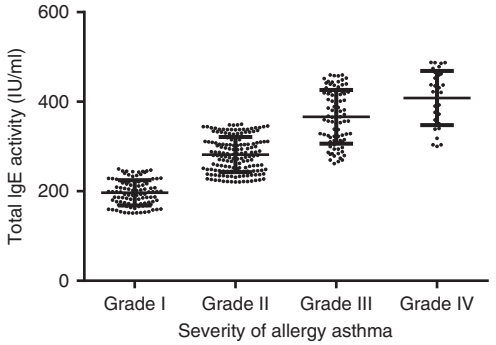

Fig. 2 The serum levels of total immunoglobulin E (IgE) in different groups. $n = 103$, 158, 86, and 31 cases with asthma grades I, II, III, and IV, respectively

Table 5. Univariate model analysis of the risks associated with atopic asthma

Characteristics	P value	Odd ratios	95% CI
Age	0.03*	0.98	0.64–1.57
Sex distribution	0.24	1.15	0.29–4.97
BMI	0.18	1.34	0.24–5.05
Birth time	0.23	0.92	0.31–2.60
Birth weight	0.46	0.98	0.65–2.23
The degree of passive smoking	0.01*	3.06	1.47–14.35
The duration of passive smoking	0.02*	2.79	0.61–5.60
Serum IgE levels	0.01*	4.220	1.65–12.23
FEV_1/FVC (%)	0.01*	4.360	1.12–8.67
FEV1 improvement (%)	0.02*	3.30	1.42–10.61
FeNO (ppb)	0.01*	4.080	0.78–8.51
Induced sputum eosinophil count (%)	0.02*	2.85	0.51–6.97

Note: There are significantly statistical differences if *$P < 0.05$
CI confidence interval, *BMI* body mass index, *IgE* immunoglobulin E, *FEV_1* forced expiratory volume in one second, *FVC* forced vital capacity, *FeNO* fractional concentration of exhaled nitric oxide

Table 6. Logistic regression—multivariate model analysis of the risks associated with asthma

Characteristics	P value	Odd ratios	95% CI
Age	0.08	0.91	0.54–1.64
The degree of passive smoking	0.01*	2.85	1.52–9.26
The duration of passive smoking	0.02*	2.58	0.72–5.32
Serum IgE levels	0.01*	3.87	1.40–9.18
FEV_1/FVC (%)	0.01*	2.97	1.56–7.31
FEV1 improvement (%)	0.02*	2.65	1.49–8.77
FeNO (ppb)	0.01*	3.42	0.98–6.32
Induced sputum eosinophil count (%)	0.03*	1.96	0.65–5.21

Note: There are significantly statistical differences if *$P < 0.05$
CI confidence interval, *IgE* immunoglobulin E, *FEV_1* forced expiratory volume in one second, *FVC* forced vital capacity, *FeNO* fractional concentration of exhaled nitric oxide

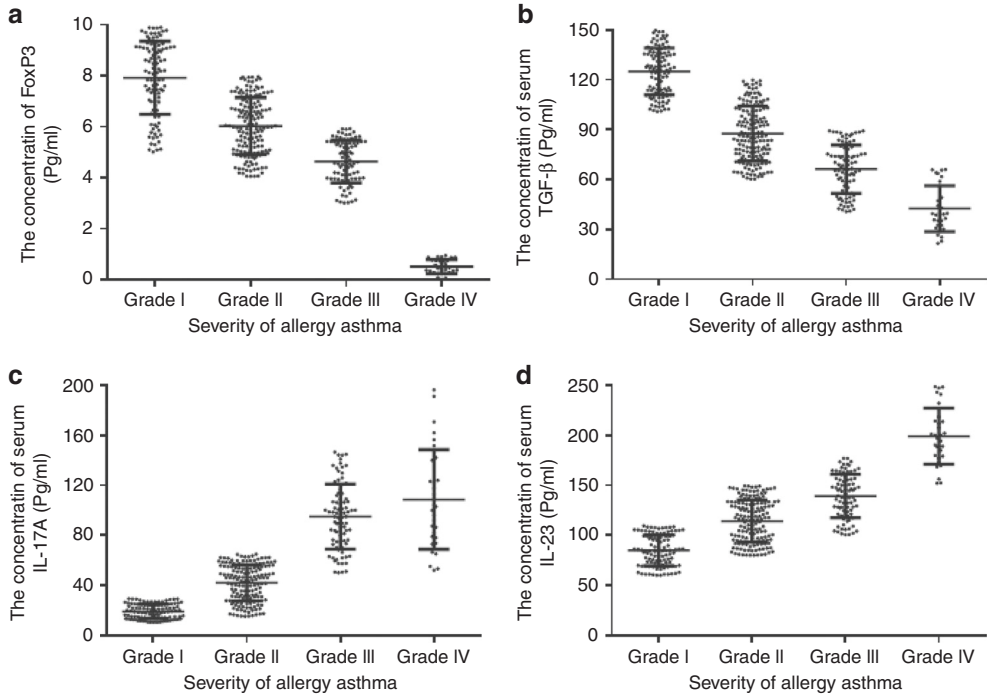

Fig. 3 The levels of cytokines in different groups. **a** The level of FoxP3 (Forkhead/winged helix transcription factor) in different groups. **b** The serum level of tumor growth factor-β (TGF-β) in different groups. **c** The serum levels of interleukin-17A (IL-17A) in different groups. **d** The serum levels of IL-23 in different groups. $n = 103$, 158, 86, and 31 cases with asthma grades I, II, III, and IV, respectively

Passive smoking reduced the contents of Treg cells and increased the contents of Th17 cells

Flow cytometry analysis showed that the percentage of Treg cells were highest in asthma grade I (Fig. 4a), high in asthma grade II (Fig. 4b), low in asthma grade III (Fig. 4c), and lowest in asthma grade IV (Fig. 4d) in the PBMCs. In contrast, the contents of Th17 cells were lowest in asthma grade I (Fig. 5a), low in asthma grade II (Fig. 5b), high in asthma grade III (Fig. 5c), and highest in asthma grade IV (Fig. 5d) in the PBMC of the patients. The results suggested that the percentage of Treg and Th17 cells were negatively and postively associated with the progression of asthma. Passive smoking aggravated the severity of asthma by affecting the balance of Treg/Th17 cells.

DISCUSSION

The impact of smoking on children has been underestimated and the research is not very thorough. In developing countries, the number of smokers is still increasing due to cigarette advertisements. The children are often exposed to high concentrations of cigarette smoke, which significantly affect their lung function. Asthma children are particularly vulnerable to passive smoking.[23] Animal experiments show that both before birth and after birth exposure (inhalation) to smoke will promote the occurrence of airway hyper-responsiveness.[24] Some research suggests that passive smoking affects the development and or worsening of childhood asthma more than the incidence of asthma without passive smoking.[25]

Children had a high risk of asthma if they were in a cigarette smoke environment. Passive smoking lead to airway inflammation in infants and young children, making it prone to viral infections, wheezing, and pneumonia.[26] Maternal smoking was a predictor of asthma in children and mothers drinking did not significantly increase their chances of developing asthma, but increased their risk by threefold if mothers smoked simultaneously.[27] If mothers smoked more than 20 cigarettes per day, the risk of asthma increased by eight times in children.[28] Maternal smoking during pregnancy lead to premature birth and low body weight,[29] and affected intrauterine fetal lung development and function. The dangers of passive smoking on asthma in children were from the perspective of pharmacokinetics and children vulnerability.[30] The asthma of the child exposed to cigarettes environment was twice as high as normal children, suggesting that passive smoking causes asthma in children.[31]

This study investigated the prevalence of passive smoking, and the results showed that the amounts and duration of passive smoking duration increased the risk of asthma in the children (Tables 5 and 6). Controlling passive smoking is very necessary in the prevention of childhood asthma. Both smoking and alcohol consumption will increase the risk of childhood asthma.

Environmental smoking is one of the risk factors for asthma in children. Moreover, previous data showed that environmental smoking increased the level of physiological stress response to inhalable allergens in asthmatic patients.[32] Treg/Th17 cell imbalance is the main cause of asthma.[33] Animal studies showed that tar, nicotine, and other toxic substances in tobacco smoke damaged the airway mucosa in a short time. Nicotine had an inhibitory effect on the release of IL-10 and IL-12 from dendritic cells in the mouse, leading to Treg/Th17 cell migration, which in turn aggravates airway inflammatory infiltration and induces asthma.[34] In the present experiment, the percentage of Th17 cells was increased, while the percentage of Treg cells was reduced (Figs. 3 and 4), and the cytokine levels were not balanced in the children with asthma caused by passive smoking (Fig. 3). The result brought new ideas for the treatment of asthma in children.

There is controversy if atopy (defined as high IgE or skin prick sensitivity) truly causes asthma. The strength of this association is under question. The term atopic disorder has been ever used to define endotypes of children with asthma, and increased IgE levels. However, an earlier report found no interdependency between asthma and allergic sensitization. Atopy in children cannot represent a true endotype of children with increased IgE levels.[35] Family history with asthma is a risk factor for childhood asthma[36] Grandparent asthma history will increase the risk of

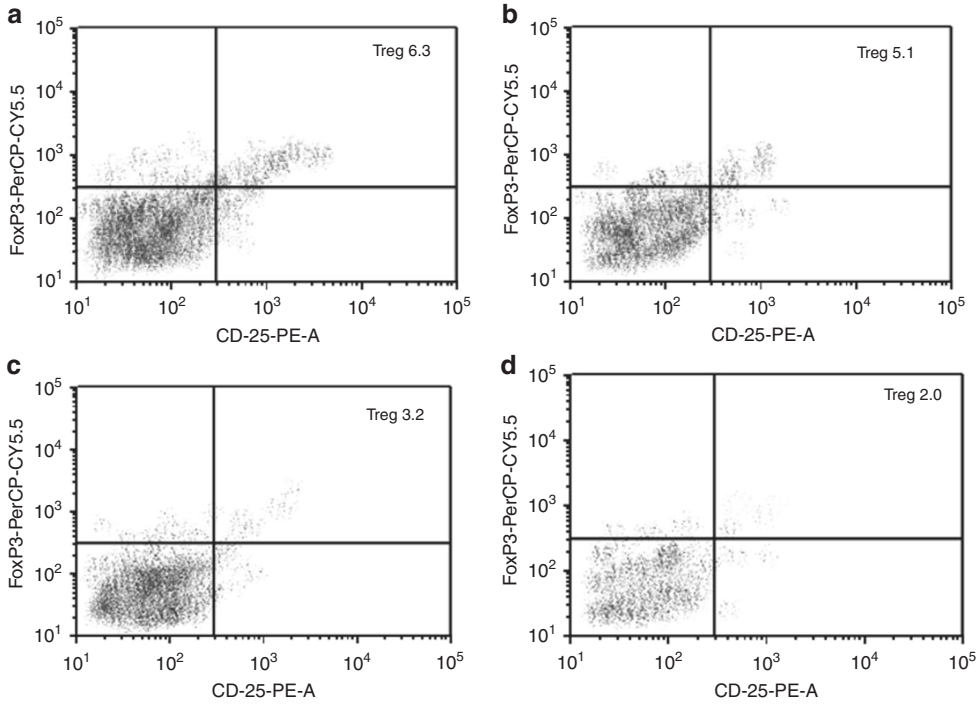

Fig. 4 The contents of T-regulatory (Treg) cells in the cases with different severity of asthma. **a** The contents of Treg cells in the cases with asthma grade I. **b** The contents of Treg cells in the cases with asthma grade II. **c** The contents of Treg cells in the cases with asthma grade III. **d** The contents of Treg cells in the cases with allergy IV. $n = 103$, 158, 86, and 31 cases with asthma grades I, II, III, and IV, respectively

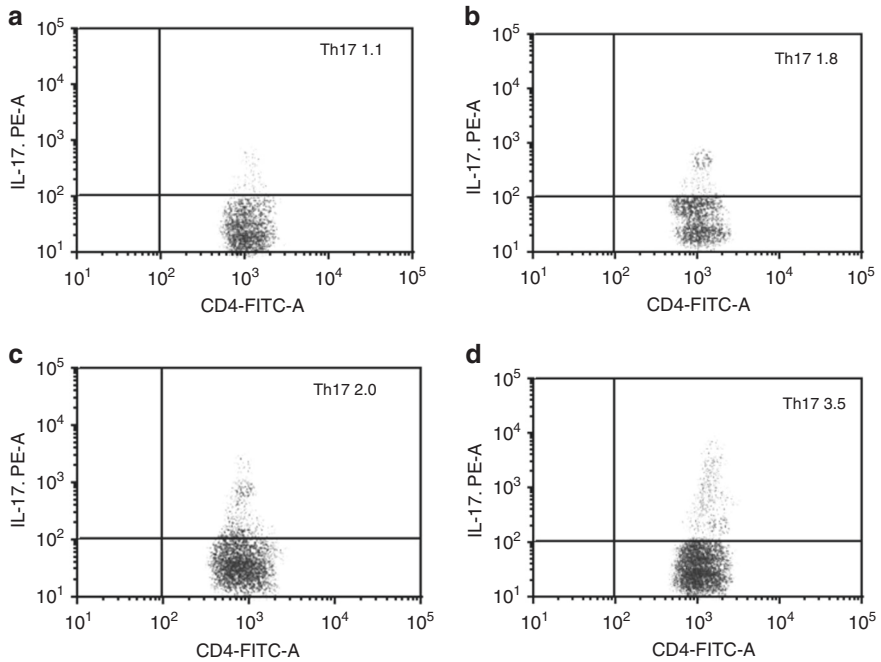

Fig. 5 The contents of T-helper type 17 (Th17) cells in the cases with different severities of asthma. **a** The contents of Th17 cells in the cases with asthma grade I. **b** The contents of Th17 cells in the cases with asthma grade II. **c** The contents of Th17 cells in the cases with asthma grade III. **d** The contents of Th17 cells in the cases with allergy IV. $n = 103$, 158, 86, and 31 cases with asthma grades I, II, III, and IV, respectively

asthma in children. Considering the risk of developing asthma in grandmas or grandmothers with asthma, the situation was excluded in the present study.

There were some limitations to the present study. Parental smoking increased the risk of childhood asthma. The risk to the child's postnatal impact in the family was mainly from the mother due to passive smoking caused by pregnant women. When mothers smoke during pregnancy or when smoking parents live in the same room with their children, their children would have an increased risk of respiratory infections, which were risk factors for

subsequent asthma. The situation was not investigated in the present experiment. Further work is highly demanded to understand the exact molecular mechanism for passive smoking causing childhood asthma.

CONCLUSIONS

In conclusion, we found an association between passive smoking and the severity of asthma in children. It was very necessary to control passive smoking in the prevention of the risk of pediatric asthma. Treg and Th17 cells were involved in the immunopathological process of asthma. Th17 cells were increased while Treg cells were reduced, and the cytokine levels were not balanced in the children with asthma, which brought new ideas for the treatment of childhood asthma.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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