

Climatic Factors Correlate with Innate Immune Response in Children with *Dermatophagoides farinae*-induced Allergic Asthma

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OBJECTIVE: To determine the effect of climatic factors on immune markers in children with *Dermatophagoides farinae*-induced asthma. **METHODS:** Serum concentrations of macrophage migration inhibitory factor (MIF), eosinophil cationic protein (ECP) and *D. farinae*-specific immunoglobulin E (DF-sIgE), together with peripheral blood eosinophil counts, were measured in children with *D. farinae*-induced ($n = 75$) or non-*D. farinae*-induced asthma ($n = 17$), and in healthy controls ($n = 30$). Mean temperature and relative humidity in the month before enrolment were calculated from meteorological data. **RESULTS:** MIF, ECP and eosinophil counts were significantly higher in children with

D. farinae-induced asthma than in controls, but comparable with non-*D. farinae*-induced asthma. Children with *D. farinae*-induced asthma in a low temperature ($< 16^{\circ}\text{C}$) or low relative humidity ($< 70\%$) climate had significantly lower DF-sIgE, MIF, ECP and eosinophil counts than those in a high temperature or high humidity climate. DF-sIgE correlated positively with MIF, ECP and eosinophil count in *D. farinae*-induced asthma. **CONCLUSIONS:** Temperature and humidity influenced MIF, ECP, eosinophil count and DF-sIgE in *D. farinae*-induced asthma. Understanding this relationship may provide new strategies for asthma prevention and treatment.

KEY WORDS: CHILDREN; ALLERGIC ASTHMA; *DERMATOPHAGOIDES FARINAE*; CLIMATIC FACTORS; MACROPHAGE MIGRATION INHIBITORY FACTOR; EOSINOPHIL CATIONIC PROTEIN

Introduction

Asthma affects a large number of children worldwide, especially in India and China.¹ Climate is an important predisposing factor for childhood asthma, allergic rhinitis and other allergic respiratory diseases, but the specific mechanisms by which climatic factors

(such as temperature and relative humidity) influence these conditions is unclear.² It is known that temperature and humidity can affect growth and reproduction of the house dust mite *Dermatophagoides farinae*, which plays a major role in allergic respiratory diseases.³ In addition, a high concentration of

D. farinae in the environment can induce acute asthma.^{4,5}

Allergic asthma involves both innate and adaptive immune responses.^{6–8} The present study investigated the relationship of ambient temperatures, relative humidity and innate immune markers associated with allergic asthma (macrophage migration inhibitory factor, eosinophil cationic protein and eosinophil count), together with *D. farinae*-specific immunoglobulin E (DF-sIgE) concentrations, in children with *D. farinae*-induced allergic asthma.

Patients and methods

STUDY POPULATION

The study sequentially enrolled children (aged ≤ 15 years) with asthma in catabasis (defined as stable asthma with no acute attacks in the previous 3 months) and an asthma control test score of > 23 points,⁹ who were attending the allergic diseases outpatient clinic of the Department of Paediatrics, Chongming Branching Hospital, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China, between September 2010 and February 2011.

Exclusion criteria included respiratory diseases other than asthma, recent upper or lower respiratory tract infections, chronic obstructive pulmonary disease, pregnancy, heart, liver and kidney diseases, diabetes, tumour history, recent surgery, systemic inflammatory disease (such as collagen vascular disease or inflammatory bowel disease) and other chronic diseases. Age- and gender-matched healthy children undergoing routine health screening at the outpatient clinic of the Department of Paediatrics, Chongming Branch Hospital, were recruited as controls.

Written informed consent was obtained from the parents or guardians of all of the study participants. The study protocol was

approved by the Ethical Review Committee of Chongming Branching Hospital, Xinhua Hospital Affiliated to Shanghai Jiaotong University School of Medicine, Shanghai, China.

ALLERGEN TEST

All study participants underwent an allergen skin-prick test (Allergopharma, affiliated company of Merck KGaA, Darmstadt, Germany), performed according to the manufacturer's instructions. Briefly, a small amount of dust mite allergen was injected intradermally on the forearm, with histamine as a positive control. The skin index (SI) was calculated as the ratio of allergen-induced wheal diameter to histamine-induced wheal diameter, with SI ≥ 0.5 considered to be positive.

LABORATORY ASSESSMENT

Venous blood was collected from all participants, incubated for 30 min at 4 °C and centrifuged at 1000 *g* for 15 min. The resulting serum was stored at -70 °C until analysed.

Serum concentrations of macrophage migration inhibitory factor (MIF), eosinophil cationic protein (ECP) and DF-sIgE were quantified by enzyme-linked immunosorbent assay (MIF and ECP, MBL International, Woburn, MA, USA; DF-sIgE, R&D Systems, Minneapolis, MN, USA) according to the manufacturers' instructions. A serum DF-sIgE concentration ≥ 0.70 IU/ml was considered positive.

An additional blood sample was collected in a sterile tube containing heparin (15 IU/ml final concentration) and stored at 4 °C until determination of eosinophil count using a Coulter STKS autoanalyser (Beckman Coulter, Brea, CA, USA).

METEOROLOGICAL DATA

Meteorological data were provided by the

Meteorological Bureau of Chongming County from over 10 automatic meteorological monitoring stations covering the Chongming district. These data were updated approximately every 1 min.

Temperature and relative humidity data were recorded daily at 02:00 h, 08:00 h, 14:00 h and 20:00 h, and daily mean values were determined. Mean temperature and relative humidity for the 30 days before enrolment were calculated for each study participant. Subjects were stratified according to mean temperature ($<$ or $\geq 16^{\circ}\text{C}$) and mean relative humidity ($<$ or $\geq 70\%$).

STATISTICAL ANALYSES

Data were presented as median (range) or mean $\pm$ SD, as indicated. Statistical significance between two independent samples was assessed using the Student's *t*-test. Multiple comparisons were performed using one-way analysis of variance, followed by Fisher's least significant difference. Relationships between parameters were assessed using Spearman's rank correlation coefficient analysis. All statistical analyses were performed using SPSS® version 15.0 (SPSS Inc., Chicago, IL, USA) for Windows®. A two-sided *P*-value < 0.05 was considered to be statistically significant.

Results

A total of 92 children with asthma were enrolled in the study, of whom 75 were considered to have *D. farinae*-induced allergic asthma on the basis of skin prick tests and serum DF-sIgE concentrations.¹⁰ The remaining 17 patients were considered to have non-*D. farinae*-induced asthma. The control group included 30 age- and gender-matched healthy subjects. Demographic characteristics of the study participants are given in Table 1.

Serum MIF and ECP concentrations, and

TABLE 1
Demographic characteristics of children with *Dermatophagoides farinae*-induced allergic asthma (DF; $n = 75$), non-*D. farinae*-induced asthma (NDF; $n = 17$) and healthy controls ($n = 30$), stratified according to mean ambient temperature and relative humidity in the 30 days before study enrolment

Characteristic	DF				NDF				Healthy controls			
	Temperature		Relative humidity		Temperature		Relative humidity		Temperature		Relative humidity	
	$< 16^{\circ}\text{C}$	$\geq 16^{\circ}\text{C}$	$< 70\%$	$\geq 70\%$	$< 16^{\circ}\text{C}$	$\geq 16^{\circ}\text{C}$	$< 70\%$	$\geq 70\%$	$< 16^{\circ}\text{C}$	$\geq 16^{\circ}\text{C}$	$< 70\%$	$\geq 70\%$
	$n = 29$	$n = 46$	$n = 18$	$n = 57$	$n = 8$	$n = 9$	$n = 7$	$n = 10$	$n = 13$	$n = 17$	$n = 14$	$n = 16$
Age, years	8	6	7	6	7	8	8	9	6	8	7	8
	(4–12)	(2–15)	(4–12)	(2–15)	(3–11)	(4–14)	(3–12)	(5–14)	(2–11)	(3–13)	(2–12)	(5–13)
Gender, male/ female	21/8	27/19	12/6	36/21	5/3	5/4	4/3	6/4	8/5	9/8	7/7	10/6

Data presented as median (range) or *n* participants.
No statistically significant differences ($P \geq 0.05$).

peripheral blood eosinophil counts, were significantly higher in patients with *D. farinae*-induced allergic asthma than in healthy children, but were comparable with levels observed in patients with non-*D. farinae*-induced asthma (Table 2). When divided according to the mean climatic conditions in the month before enrolment, patients with *D. farinae*-induced allergic asthma in the $< 16^{\circ}\text{C}$ temperature and $< 70\%$ relative humidity groups had significantly lower values for MIF, ECP and eosinophil counts than the corresponding higher temperature and humidity groups. MIF, ECP and eosinophil counts in patients with non-*D. farinae*-induced asthma and in healthy controls were not affected by temperature or relative humidity. In patients with *D. farinae*-induced allergic asthma, DF-sIgE concentrations were significantly lower in the low temperature group than in the high temperature group.

There were significant positive correlations between concentrations of DF-sIgE and MIF (Fig. 1A), ECP (Fig. 1B) and eosinophil count (Fig. 1C) ($P < 0.01$ for all correlations). There were also significant positive correlations between MIF and ECP ($P = 0.005$), MIF and eosinophil count ($P = 0.001$), and ECP and eosinophil count ($P = 0.001$).

Discussion

Allergic asthma, allergic rhinitis and other respiratory diseases are characterized by the occurrence of seasonal morbidity, which is associated with climatic factors such as ambient temperature and relative humidity.¹¹ Climatic factors may affect the growth and reproduction of *D. farinae*, which is regarded as having a major role in allergic respiratory diseases.² Climatic factors (ambient temperature and relative humidity) correlated with the innate

immune response to *D. farinae* in the present study. Higher mean temperature and relative humidity were associated with higher concentrations of DF-sIgE in patients with *D. farinae*-induced allergic asthma. In addition, DF-sIgE levels in these patients correlated positively with MIF, ECP and eosinophil counts.

A dose-effect relationship between severity of asthma symptoms and household *D. farinae* allergen content has been reported,^{12–14} and the prevalence of asthma has been shown to be associated with seasonal variations in the concentration of *D. farinae* allergen in the environment.^{15,16} Growth and reproduction of *D. farinae* – which is a major allergen associated with respiratory allergic diseases in the Chongming district of China¹⁵ – requires specific temperature and humidity conditions.^{13–15} High mean temperature and high relative humidity during the 30 days before enrolment was associated with a higher level of DF-sIgE than low mean temperature and low relative humidity in the present study. As DF-sIgE is an important marker for allergy to *D. farinae*, this suggests that a higher reproduction rate for *D. farinae* at optimum temperature and relative humidity¹³ may have induced a concurrent increase in the generation of *D. farinae*-specific antibodies. The wide distribution of the patients involved and the lack of effective technology for accurately detecting environmental dust mites rendered the direct collection of dust mites or their detritus unfeasible in the present study.

Bronchial asthma is a chronic inflammatory airway disease, involving various cell types such as eosinophils, mast cells, T cells, neutrophils and airway epithelium cells. Eosinophils are one of the most important initial inflammatory cells in allergic diseases and play important roles in

TABLE 2: Peripheral blood eosinophil counts and serum concentrations of macrophage migration inhibitory factor (MIF), eosinophil cationic protein (ECP) and *Dermatophagoides farinae*-specific immunoglobulin E (DF-sIgE), in children with *D. farinae*-induced allergic asthma (DF), children with non-*D. farinae*-induced asthma (NDF) and healthy controls, stratified according to mean ambient temperature and relative humidity in the 30 days before study enrolment

Parameter	DF n = 75		NDF n = 17		Healthy controls n = 30	
	< 16 °C n = 29	≥ 16 °C n = 46	< 16 °C n = 8	≥ 16 °C n = 9	< 16 °C n = 13	≥ 16 °C n = 17
Serum MIF, ng/ml		6.83 ± 2.36 ^a		7.19 ± 1.37 ^a		0.63 ± 0.31
Serum ECP, ng/ml		3.42 ± 0.96 ^a		3.74 ± 1.18 ^a		2.99 ± 0.92
Eosinophil count, × 10 ⁹ /l		0.63 ± 0.31 ^a		0.59 ± 0.26 ^a		0.27 ± 0.10
Serum DF-sIgE, IU/ml		6.53 ± 3.10		0.6 ± 0.25		NA
Ambient temperature						
Serum MIF, ng/ml	5.30 ± 1.35 ^{b,c}	7.80 ± 2.36 ^{a,b}	7.01 ± 1.00 ^a	7.35 ± 1.41 ^a	0.60 ± 0.29	0.65 ± 0.34
Serum ECP, ng/ml	2.97 ± 0.59 ^{b,c}	3.70 ± 1.05 ^a	3.68 ± 1.08 ^a	4.26 ± 1.30 ^a	2.95 ± 0.88	3.05 ± 1.02
Eosinophil count, × 10 ⁹ /l	0.52 ± 0.22 ^{a,c}	0.70 ± 0.34 ^{a,b}	0.60 ± 0.25 ^a	0.59 ± 0.33 ^a	0.26 ± 0.10	0.28 ± 0.11
Serum DF-sIgE, IU/ml	5.54 ± 1.60 ^c	7.16 ± 3.64	NA	NA	NA	NA
Relative humidity	< 70% n = 18	≥ 70% n = 57	< 70% n = 7	≥ 70% n = 10	< 70% n = 14	≥ 70% n = 16
Serum MIF, ng/ml	5.36 ± 1.37 ^{a,b,d}	8.30 ± 2.42 ^a	7.10 ± 1.33 ^a	7.27 ± 1.40 ^a	0.61 ± 0.30	0.64 ± 0.36
Serum ECP, ng/ml	3.11 ± 0.58 ^{a,b,d}	3.91 ± 1.04 ^a	3.70 ± 1.11 ^a	3.78 ± 1.28 ^a	2.97 ± 0.90	3.02 ± 0.98
Eosinophil count, × 10 ⁹ /l	0.55 ± 0.24 ^{a,d}	0.66 ± 0.33 ^a	0.59 ± 0.24 ^a	0.60 ± 0.32 ^a	0.26 ± 0.09	0.28 ± 0.21
Serum DF-sIgE, IU/ml	5.59 ± 1.81	6.83 ± 3.37	NA	NA	NA	NA
Data presented as mean ± SD.						
NA, data not available.						
^a p < 0.05 compared with healthy controls; one way analysis of variance (ANOVA) followed by Fisher's least significant difference test (LSD).						
^b p < 0.05 compared with NDF; ANOVA followed by LSD.						
^c p < 0.05 compared with ≥ 16 °C within group; Student's <i>t</i> -test.						
^d p < 0.05 compared with ≥ 70% relative humidity within group; Student's <i>t</i> -test.						

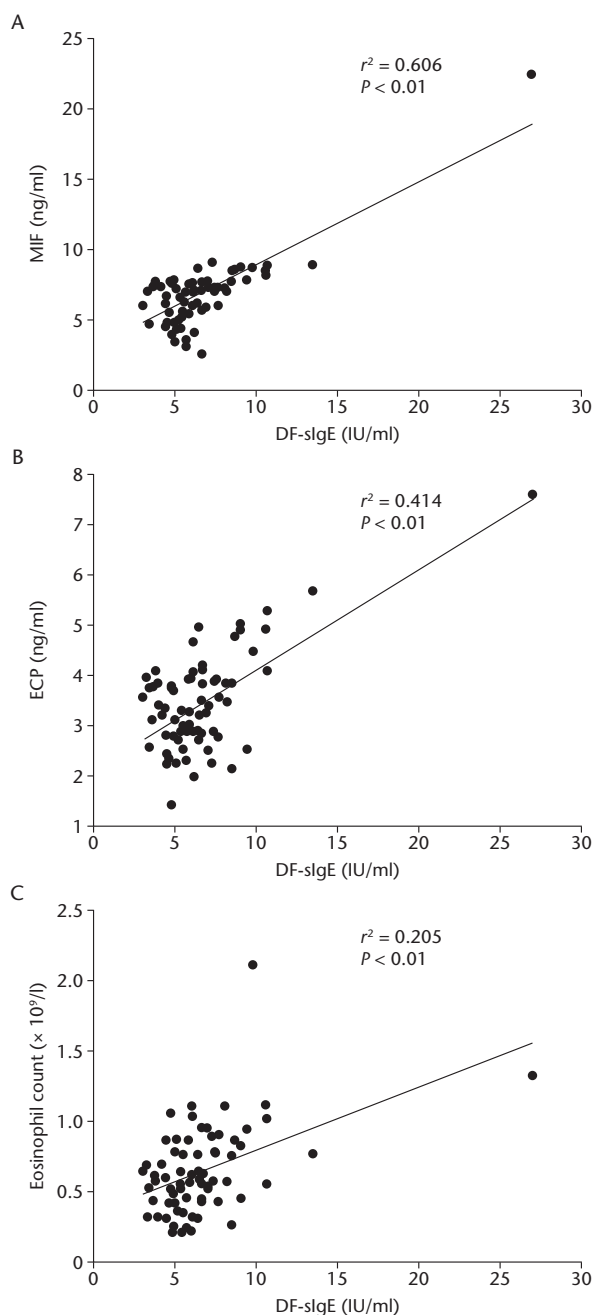


FIGURE 1: Correlations between *Dermatophagoides farinae*-specific immunoglobulin E (DF-slgE) concentration and: (A) serum macrophage migration inhibitory factor (MIF) concentration; (B) serum eosinophil cationic protein (ECP) concentration; (C) peripheral blood eosinophil count in children with *D. farinae*-induced allergic asthma ($n = 75$). $P < 0.01$ for all correlations, Spearman's rank correlation coefficient

the pathogenesis of asthma.¹⁷ ECP is an important indicator of eosinophil activation,¹⁸ and serum ECP levels have been shown to be increased in children with allergic rhinitis or asthma compared with healthy children, and in acute asthma compared with asthma in catabasis.⁷ Eosinophil count and ECP level may, therefore, reflect the status of allergic inflammation.⁸ As a proinflammatory factor, MIF inhibits migration of macrophages and regulates T cell proliferation and activation; it also plays a regulatory role in chronic inflammation.^{19,20} Eosinophils are an important source of MIF, especially in asthma patients.²¹ Moreover, MIF and ECP have been shown synergistically to promote eosinophils to gather at inflammation sites and induce synthesis of leucotriene C₄, leading to allergen-induced allergic inflammation.²² MIF plays an important role in the pathogenesis of allergen-induced asthma.²² In the present study, in children with *D. farinae*-induced allergic asthma living in warmer climates ($\geq 16^{\circ}\text{C}$), serum MIF and ECP concentrations, together with eosinophil counts, were higher than the levels observed in children with *D. farinae*-induced asthma who were living in cooler climates. A similar finding was observed in children in climates with high relative humidity ($\geq 70\%$) compared with those living in climates with low relative humidity.

It has been reported that the severity of allergic inflammation in children with *D. farinae*-induced allergic asthma correlates with ambient temperature and relative humidity.¹⁻³ In contrast, climatic factors play a lesser role in non-*D. farinae*-induced asthma, since the causative factors are usually infections (including endotoxins) or food allergies.² This is consistent with the results of the present study, in which the serum MIF and ECP concentrations – and

eosinophil counts – in patients with non-*D. farinae*-induced asthma were unaffected by temperature or relative humidity.

Allergic inflammation in children with *D. farinae*-induced allergic asthma is necessarily dependent on the presence of allergens from *D. farinae*.^{1,2} Serum MIF, ECP and eosinophil counts have been shown to be important inflammatory factors in the initial phase of allergic inflammation in asthma.²¹⁻²³ Magalhães *et al.*²⁰ found that MIF was closely associated with eosinophils, both of which play important roles in the progression of asthma.²³ Positive correlations were found between concentrations of DF-sIgE, MIF and ECP, and eosinophil count, in the children with *D. farinae*-induced allergic asthma in the present study.

In conclusion, the present study found a relationship between temperature and relative humidity and the innate immune response markers MIF, ECP and eosinophil count, in children with *D. farinae*-induced allergic asthma, but not in children with non-*D. farinae*-induced asthma. In addition, in children with *D. farinae*-induced allergic asthma, the specific marker DF-sIgE correlated with the inflammatory factors MIF, ECP and eosinophil counts, as well as with ambient temperature and relative humidity. These findings are helpful for understanding the relationship between climatic factors and *D. farinae*-induced allergic asthma, and may provide new strategies for asthma prevention and treatment.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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