

Quantification of circulating house dust mite-specific IL-4- and IL-13-secreting T cells correlates with rhinitis severity in asthmatic children and varies with the seasons

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Summary

Background Defining suitable markers to diagnose and monitor allergy and its severity is essential to correctly assign patients for specific immunotherapy. Circulating levels of specific IgE are good markers of sensitization, but not of clinically symptomatic allergy.

Objective To quantify circulating interleukin (IL)-4- and IL-13-secreting T cells specific for house dust mite (HDM) in children presenting HDM-allergic asthma associated or not with rhinitis and correlate results with clinical symptoms.

Methods We analysed 26 children with HDM respiratory disease (allergic rhinitis and asthma) together with six children with non-allergic asthma. Peripheral blood mononuclear cells were stimulated with HDM extract in a 24-h ELISpot assay to quantify the number of HDM-specific IL-4- and IL-13-secreting T cells. Asthma severity and control, and rhinitis severity were scored according to the Global Initiative for Asthma (GINA) and the Allergic Rhinitis and its Impact on Asthma (ARIA) Guidelines.

Results The number of HDM-specific IL-4- and IL-13-secreting T cells was higher in patients with allergic asthma as compared to patients with non-allergic asthma. It varied with the season of blood sampling with two peaks in the fall and early spring. Independently of the season, the number of HDM-specific IL-4-secreting T cells correlated with rhinitis severity (OR = 2; 95% IC:1.1–3.8; $P = 0.04$).

Conclusions and Clinical relevance Allergen-specific IL-4- and IL-13-producing T cells were only detected in HDM-allergic asthmatic children (not in patients with non-allergic asthma). Their numbers correlated with clinical severity of allergic rhinitis.

Keywords allergic rhinitis, asthma, house dust mite, IL-4, IL-13, Th2 lymphocytes

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Introduction

The prevalence of allergic diseases is steadily increasing world-wide and represents a significant public health problem especially in industrialized countries [1–3]. In European countries among the inhaled allergens, house dust mite (HDM) is the most common perennial allergen responsible for symptomatic manifestations of allergy ranging from allergic rhinitis to severe asthma [4]. Specific allergen immunotherapy is the only specific treatment, which provides a real cure for the disease [5]. This specific treatment for allergy also allows the

improvement of a pre-existent asthma and prevents the development of new sensitizations [6, 7].

Allergic polysensitization is a frequent phenotype of allergic asthma [8]. Hence, the importance of identifying the major allergen responsible for the symptoms before starting specific allergen immunotherapy is a major issue. In this situation, defining suitable and easy-handling antigen-specific T cell biomarkers would therefore considerably improve patients' management and targeted treatment such as specific allergen immunotherapy.

In fact, currently, the bronchial provocation test and nasal provocation test are the gold standards for

diagnosis of mite allergy. However, these are invasive tests, presenting well-known risks that must be carried out in a specialized environment. Among immunological tests, routinely used is the determination of specific immunoglobulin E (IgE) that reflects the sensitization to an allergen, but is not satisfactory for the diagnosis of mite allergy. In addition, the recently developed basophil activation test is a functional readout, which, in terms of diagnosis, has not yet proved to be superior in the diagnosis of mite allergy compared with the dosage of specific IgE [9, 10]. Type 2 helper T lymphocytes (Th2) play a central role in orchestrating the inflammatory response in allergy through their secretion of interleukin (IL)-4 and IL-13 mediating B cell stimulation and IgE synthesis [11, 12].

The enzyme-linked immunospot (ELISpot) assay is a sensitive tool for enumerating cells producing a given cytokine following stimulation with an antigen or a mitogen. The ELISpot assay was described 25 years ago initially for the detection of interferon (IFN) γ production at the single cell level [13]. The reliability of the IFN γ ELISpot in quantifying antigen-specific T cells has been validated by several laboratories including ours, in the monitoring of a wide variety of indications, including patients with infectious [14, 15], autoimmune and neoplastic diseases undergoing immunotherapeutic treatments [16].

The advantages of the ELISpot assay are the capacity to identify antigen-specific cells, producing a given cytokine, to do this at the single cell level, therefore allowing the quantification of responding cells and, last but not least, to measure responses following a short-term *in vitro* culture. Indeed, it has been demonstrated that such short-term stimulation allows the detection of already differentiated antigen-specific cells including the *in vivo* activated effector and/or memory T cell pool. Therefore, the measured response visualizes the *in vivo* cellular number of circulating differentiated antigen-specific T cells in an individual. In this study, we enumerated the number of HDM-specific IL-4- and IL-13-producing T lymphocytes in a series of consecutive clinically well-characterized asthmatic children presenting varying degrees of allergic rhinitis.

Methods

Patient population

This was a cross-sectional study, and blood samples were collected once for each patient between January 2011 and May 2012. We wanted to evaluate ELISpot responses depending on the seasons during 1 year. Therefore, among the whole cohort of 26 patients, 17 children were sampled once between January 2011 and November 2011, analysed and compared one to

another. The patients we analysed were part of the 2 years (2011–2012) Trousseau Asthma Program at the Hôpital Trousseau in Paris.

Inclusion criteria for the allergic group were as follows: 1) age higher than 5 years, 2) diagnosis of active asthma (defined as a history of recurrent wheeze or more than three episodes of reversible bronchial obstruction documented within the previous 6 months), 3) absence of other chronic obstructive pulmonary diseases (congenital or acquired), 4) *Allergic rhinitis*, assessed using the questionnaire from the International Study of Asthma and Allergies in Childhood (ISAAC) [17] and asking the questions: a. Has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu? b. In the past 12 months, has your child had a problem with sneezing, or a runny, or a blocked nose when he/she did not have a cold or the flu? c. In which of the past 12 months did this nose problem occur? Rhinitis severity was determined according to the World Health Organization (WHO) ARIA (*Allergic Rhinitis and its Impact on Asthma*) Guidelines, based on the impact of rhinitis upon patient quality of life [18]. Rhinitis was classified as mild intermittent, mild persistent, moderate persistent and severe persistent. No child exhibited moderate/severe intermittent rhinitis. To perform statistical analysis, mild intermittent and mild persistent rhinitis were grouped on the one hand, and moderate persistent and severe persistent rhinitis were grouped on the other hand. 5) presence of allergic sensitization to HDM [defined as positive skin prick test (weal ≥ 3 mm compared with negative control) and specific IgE ≥ 0.35 kU/L against *Dermatophagoides pteronyssinus*] in absence of sensitization to perennial allergens (mould, cockroach, cat and dog) and of clinical symptoms of seasonal allergy (four patients were sensitized to grass pollen (skin prick and sIgE) and three to grass pollen and birch pollen (skin prick and sIgE); however, none of them had conjunctival symptoms during pollen season), 6) the achievement of clinical examination outside of episodes of exacerbation or acute respiratory illness before initiation of specific immunotherapy 7) and finally having no previous specific immunotherapy.

Inclusion criteria for the non-allergic group: Patients presenting non-allergic asthma who featured the same clinical characteristics of the allergic group except that they did not have any symptoms of allergic rhinitis, and they did not present any allergic sensitization to HDM [skin prick test and specific IgE towards *Dermatophagoides pteronyssinus*] nor sensitization to perennial allergens (mould, cockroach, cat and dog) or seasonal allergy (birch and grass pollen).

Asthma was defined as intermittent, mild persistent, moderate persistent or severe persistent according to the Global Initiative for Asthma (GINA) 2005 [19]. All

enrolled children were treated with fixed dose of inhaled corticosteroids (ICS) for at least 6 months and were compliant with their prescribed treatment. High doses of ICS were defined as 500 µg or more of fluticasone (or its equivalent) per day. The control of asthma was assessed according to GINA 2009 and to Asthma Control Test (ACT) [20]. The outcome of treatment was ranked as follows: controlled with or without high-dose ICS or uncontrolled (including partially controlled) with or without high-dose of ICS.

The study protocol was approved by the Paris 5 ethics committee, and all patients gave written informed consent.

ELISpot assay

Blood samples were collected in citrate phosphate dextrose adenine (CPDA)-pre-treated blood collection tubes. They were stored at room temperature and processed within 24 h following collection. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation. Ninety-six well PVDF (Millipore) plates were permeabilized for 1 min using 35% ethanol, washed five times with PBS and coated overnight at 4°C with antibodies to interleukin (IL)-4 or IL-13 following the manufacturer's instructions (U-Cytech, Utrecht, the Netherlands). Then, the plates were incubated for 1 h at 37°C with blocking solution and washed 3 times with PBS. PBMC were resuspended in culture medium (RPMI 1640 from Invitrogen containing L-glutamine and supplemented with penicillin, streptomycin and 10% foetal calf serum) and added at 0.3×10^6 per well in triplicates to coated plates together with HDM (from *Dermatophagoides pteronyssinus*) or phytohemagglutinin as positive control (PHA, 10 µg/mL, DIFCO) or medium alone as negative control.

HDM extract was obtained from Stallergene, France, at a concentration corresponding to 20 µg/mL Derp 1, a major allergen of *Dermatophagoides pteronyssinus*. In all tests, the preparation used is the clinical grade material provided for *in vivo* prick tests manufactured to avoid endotoxin-linked skin inflammatory reactions.

After culture for 20 h at 37°C, the cells were removed by extensive washing, and a secondary antibody to IL-4 or IL-13 (U-Cytech) was added and incubated for 1 h at 37°C. After extensive washing of both sides of the plate, GABA (phi-labelled antibiotin antibodies) conjugate (U-Cytech) was added for 1 h at 37°C. After another series of washings, ActivatorII was added to visualize spots. The reaction was stopped 25 min later by washing with water, and plates were air-dried for 1 h before spot counting using an ELISpot reader (Autoimmun Diagnostika, Strassberg, Germany).

All data shown are means of triplicate wells and expressed as spot-forming cells per 0.3×10^6 lymphocytes.

Total and specific IgE levels

Serum levels of total immunoglobulin E (IgE) and specific antibodies (IgE, IgG4) against *Dermatophagoides pteronyssinus* were determined in whole blood using the immunoCAP-System (Thermo Fischer, Uppsala, Sweden).

Statistical analysis

Statistical analysis was performed with GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Intergroup comparisons of continuous variables were performed with the Mann–Whitney *U*-test, and bivariate correlation was analysed with the Spearman's rank test. A multiple logistic regression analysis was used to assess the strength of the statistical associations in the presence of confounding factors, with STATA 12.1 software (Stanford IT services, Stanford, CA, USA). Two-sided *P*-values of <0.05 were considered statistically significant.

Results

Patients and controls characteristics

This was a cross-sectional study that included 26 consecutive asthmatic patients who met the inclusion criteria, before undergoing specific immunotherapy to HDM. Twenty-one were boys (80.8%), and mean age was 9.14 (± 2.85) years (mean $\pm$ SD). Most of the children had moderate to severe asthma ($n = 20$, 77%). Patients with intermittent to mild persistent rhinitis and patients with moderate to severe persistent rhinitis were evenly distributed (see Table 1). Most of them were monosensitized to HDM ($n = 15$, 58%). Six patients were included in the non-allergic group; mean age was 9.1 (± 4.3) years. Most of them had severe asthma ($n = 5$, 83%). None had rhinitis.

The number of HDM-specific IL-4- and IL-13-secreting T cells is associated with the presence of HDM respiratory allergy

In a set of preliminary experiments using peripheral blood cells recovered from normal healthy donors, we confirmed that the HDM preparation used did not trigger a non-specific IL-4 or IL-13 production by T cells. Phytohemagglutinin (PHA) was used in these and in the further experiments as a positive control showing over 300 spots for 0.3 million PBMC for IL-4 and IL-13.

In patients sensitized to HDM, the number of cytokine producing T cells following HDM stimulation detected by

Table 1. Subjects' characteristics

	Allergic group	Non-allergic group
Number of patients	26	6
Gender (male/female)	21/5	4/2
Age (years) [§]	9.14 ($\pm$ 2.85)	9.05 ($\pm$ 4.3)
Intermittent to mild persistent allergic rhinitis [¶]	13 (50)	0
Moderate to severe rhinitis [¶]	13 (50)	0
Controlled asthma with low doses ICS [¶]	15 (58)	0
Uncontrolled asthma with high doses ICS [¶]	4 (15)	3 (50%)
Total IgE (kU/L) [‡]	294 (135.5–653.5)*	191.5 (30.5–343)
HDM-specific IgE (kU/L) [‡]	70 (28–100)*	0
Derp1-specific IgE (kU/L) [‡]	34.9 (6.7–84.7)*	–
Derp1-specific IgG4 (mgA/L) [‡]	0.3 (0.20–0.88) [†]	–

*n = 25,

†n = 24.

Values are expressed as †median (interquartile range) or

§mean ($\pm$ standard deviation) or ¶number (%).

ELISpot ranged from 0 to 85.2 spots over 0.3 million PBMC for IL-4. Half of them had more than 32 spots. It ranged from 0 to 68.7 spots for IL-13. Half of them had more than 33 spots (see Fig. 1). As also shown in Fig. 1, the two responses were highly correlated ($P < 0.0001$, $r = 0.88$). In contrast, in the non-allergic group (including patients presenting with non-allergic asthma), we never detected any HDM-specific T cell response neither for IL-4 nor for IL-13 (see Figs 1 and 2). The number of HDM-specific IL-4-producing T cells was 32.15 (11–60.03) (median IQR) in patients as compared to 0 in controls ($P = 0.0005$). The number of HDM-specific IL-13 producing T cells was 33.25 (14.9–50.4) in patients as compared to 0 in controls ($P = 0.0005$). In both groups, the number of specific T cells in response to Phytohemagglutinin (PHA) was over 300 spots for 0.3 million PBMC for IL-4 and IL-13.

The number of IL-4- and IL-13-secreting HDM-specific T cells varied with the seasons

During the study, we found that the number of IL-4- and IL-13-secreting T cells in response to HDM significantly varied depending on the season of sampling and showed two periods (see Fig. 3). In those 17 patients tested, an intense response was observed during the fall and early spring compared with a significantly milder response during winter and summer ($P = 0.002$ for IL-4 and $P = 0.008$ for IL-13). Therefore, for the multivariate analysis, we categorized as season with a high response (the fall and early spring) and season with a low response (winter and summer).

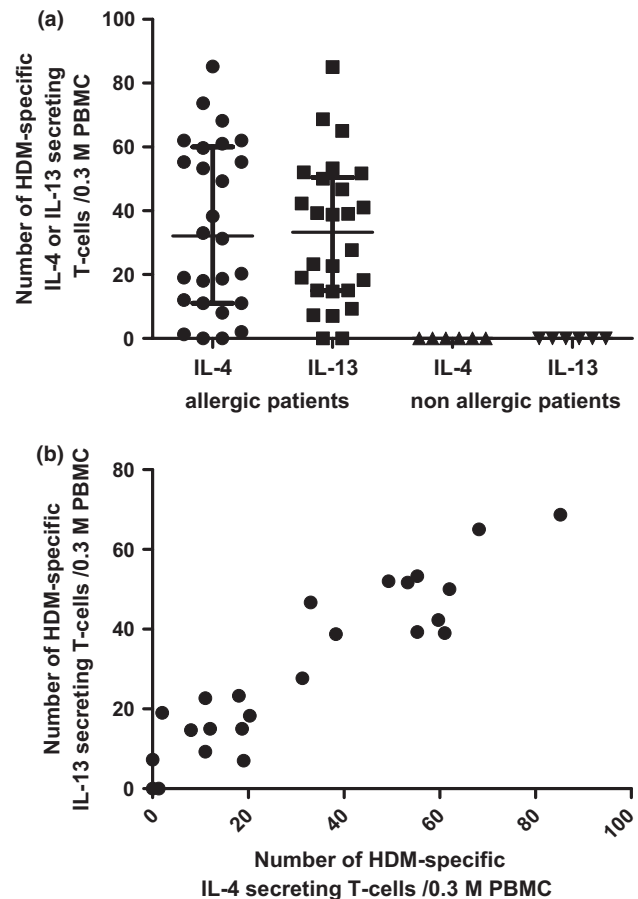


Fig. 1. (a) Number of house dust mite (HDM)-specific interleukin (IL)-13- and IL-4-secreting T cells in allergic and non-allergic patients. Median is shown with interquartile range. (b) Correlation diagram between the HDM-specific IL-13- and IL-4-secreting T cells. $r = 0.88$ $P < 0.0001$

The number of HDM-specific IL-4 secreting T cells correlates with the severity of allergic rhinitis

Allergic rhinitis severity was categorized as intermittent to mild persistent or moderate to severe persistent. As shown in Fig. 4, we found that the number of IL-4-secreting HDM-specific T cells significantly differed with the severity of rhinitis. Thus, children with moderate to severe persistent rhinitis had a higher number of IL-4 secreting HDM-specific T cells than those presenting intermittent to mild persistent rhinitis ($P = 0.04$, see Fig. 4). The number of IL-13-secreting HDM-specific T cells also tended to be higher in children with moderate to severe persistent rhinitis than in children with intermittent to mild persistent rhinitis ($P = 0.06$, see Fig. 4). In a univariate analysis, neither asthma control or severity nor HDM sensitization (specific IgE) or total IgE was associated with rhinitis severity (see Table 2) [21, 22].

We used a logistic regression model. Odd ratios are given for a variation of 10 HDM-specific IL-4- or

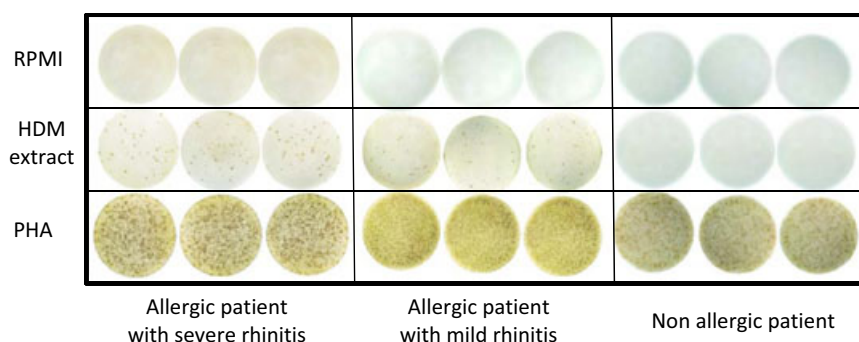


Fig. 2. Representative raw data of the ELISpot in three different patients. 0.3×10^3 PBMC were plated in each well coated with anti-IL-4 antibody, in triplicates. PHA was used as positive control, RPMI as negative control and HDM extract as antigen. Each spot represents a single IL-4-secreting T cell. 68 HDM-specific IL-4-secreting T cells for 0.3×10^3 PBMC were detected in the patient with severe rhinitis, 31 HDM-specific IL-4-secreting T cells for 0.3×10^3 PBMC in the patient with mild rhinitis and none in the non-allergic patient.

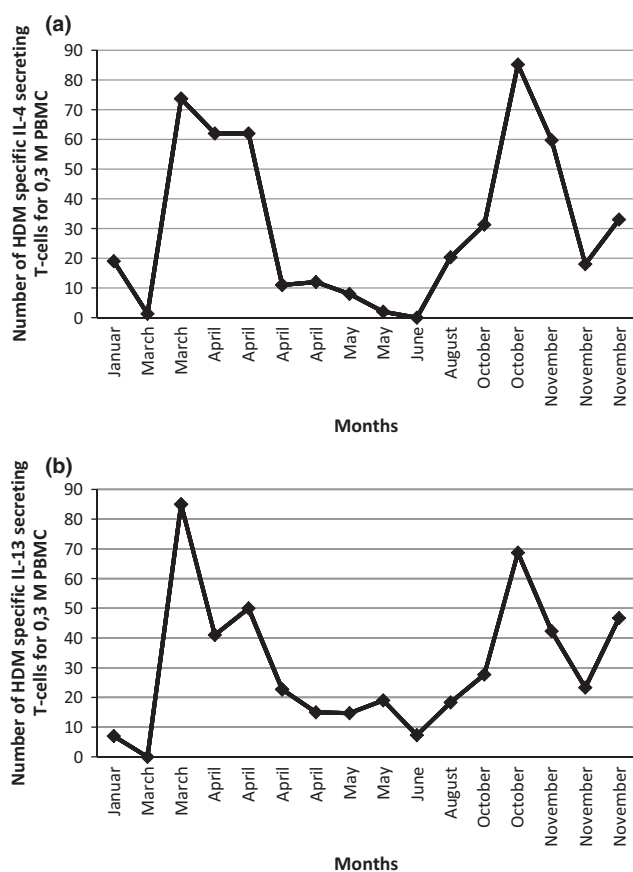


Fig. 3. House dust mite-specific IL-4 (a)- or IL-13 (b)-secreting T cells fluctuate with the seasons with two peaks in the fall and in early spring. 17 patients were tested once between January 2011 and November 2011. Each date represents one patient's sample of the 17 patients' sample.

IL-13-secreting T cells. Data were adjusted for age and gender as possible confounding factors. Because specific T cell response varied depending on the season, we also adjusted for season of blood sampling as possible confounding factor. Seasons of blood sampling were categorized as season with high response (the fall and

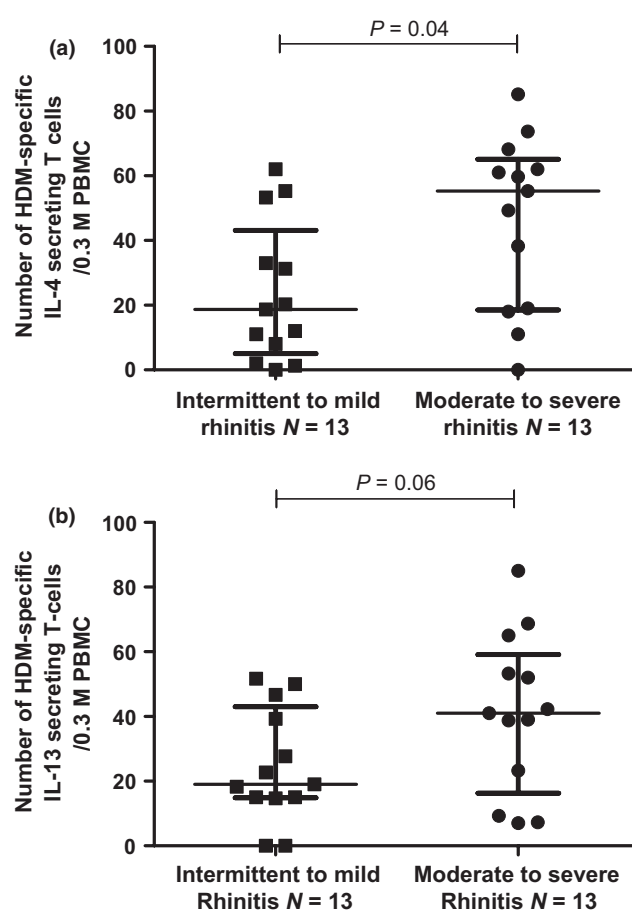


Fig. 4. House dust mite-specific IL-4 (a)- or IL-13 (b)-secreting T cells related to rhinitis severity. Median is shown with interquartile range.

early spring) and season with a low response (winter and summer). Seasons were arbitrarily determined depending on ELISpot responses.

The severity of the rhinitis was determined by the number of IL-4-secreting HDM-specific T cells (OR: 1.46; 95%IC 1.03–2.08; $P = 0.04$). This association was also found after adjusting for age, gender or season.

Table 2. Associations between the severity of the rhinitis and asthma control, asthma severity, HDM sensitization and ELISpot results

	OR (95%IC)	<i>P</i>				
Univariate analysis						
Number of HDM-specific IL-4-secreting T cells	1.5 (1.03–2.08)	0.04				
Number of HDM-specific IL-13-secreting T cells	1.5 (0.96–2.22)	0.07				
Total IgE	1 (0.95–1.0)	0.1				
HDM-specific IgE	1 (0.86–1.27)	0.7				
Specific IgE to Derp1	0.9 (0.74–1.12)	0.4				
Asthma control	1.1 (0.57–2.25)	0.7				
Asthma severity	1.7 (0.66–4.26)	0.3				
	Adjusted for season	Adjusted for gender	Adjusted for age			
	OR (95%IC)	<i>P</i>	OR (95%IC)	<i>P</i>		
Multivariate analysis. Analyses were adjusted for season of blood sampling, gender or age as possible confounding factors						
Number of HDM-specific IL-4-secreting T cells	2 (1.05–3.83)	0.04	1.8 (1.09–2.8)	0.02	1.5 (1.02–2.08)	0.04
Number of HDM-specific IL-13-secreting T cells	1.7 (0.94–2.92)	0.07	1.7 (1.04–2.7)	0.03	1.5 (0.95–2.22)	0.08

The severity of the rhinitis also tended to be associated with the number of IL-13-secreting HDM T cells (OR = 1.5; 95%IC 0.96–2.2; $P = 0.07$).

Discussion

With the development of allergen-specific immunotherapy and the increasing polysensitizations, it has become extremely important to accurately identify the allergen that triggers the clinical symptoms. The biological parameter commonly used to diagnose HDM respiratory allergy is the serum level of specific IgE, which is certainly a good marker for sensitization, but not for the clinical symptoms and therefore for allergy. Biological functional tests represent an interesting alternative. A positive correlation was shown between HDM-specific basophil activation and HDM-specific IgE levels, but a relationship with the clinical symptoms has not yet been demonstrated [9]. HDM-specific Th2 responses were described to correlate with various aspects of allergic disease (HDM-specific IgE, skin prick test positivity, non-specific bronchial hyper reactivity) [23–26]. Of interest are the data showing that the Th2 response namely, IL-5 secretion by T cells cultured in presence of HDM correlated with the response to the bronchial provocation test to HDM that is the gold standard for the diagnosis of allergy [27].

Here, we quantitated allergen-specific IL-4- and IL-13-secreting T cells, using an ELISpot after a short 20 h *in vitro* stimulation, in HDM-allergic asthmatic children. Our current data demonstrate that with this method, it is possible to distinguish HDM-allergic asthmatic children from patients presenting with non-allergic asthma. IL-4 and IL-13 responses were significantly correlated; studies are in progress to evaluate the proportion of T cells producing the two cytokines.

It is important to highlight three technical factors that appear crucial to explain the greater specificity of this test compared with those previously described in the literature [23–29]. The first is the use of a crude preparation of allergen which, when compared to the use of purified recombinant allergens or their derivate peptides, allows evidencing a response to a larger number of antigenic determinants and thereby uncovering the response in a larger number of patients. Using this crude preparation, no response was detected in the non-allergic group. However, it is fair to admit that this result needs confirmation as only six children were included in this group. The second factor is the short *in vitro* stimulation used (i.e. 20 h) that can be applied due to the high sensitivity of ELISpot and which improves the final specificity of the read out as it favours the detection of experienced activated and memory T cells, thereby representing a snapshot of the *in vivo* existing situation. This has been initially demonstrated in individuals after recovery from a viral infection who exhibit low frequency of specific T cells undetectable by conventional methods without a previous *in vitro* expansion with cognate antigen which may introduce qualitative and quantitative biases [30]. In the particular case of influenza virus because of the high sensitivity of the short-term stimulation ELISpot, it was possible to detect circulating CD8⁺ memory T cells capable of rapid effector function [30].

Third, in terms of read out what the ELISpot provides is a quantification of allergen-specific T cells as compared to the measurement of cytokine levels in the supernatants of T cell cultures [31, 32]. The only other way to track antigen-specific T cells is the use of HLA-peptide-tetramers directed to the allergen-specific T cell receptors that have been applied in different kind of allergies (peanut, wasp venom, cockroach, dog, pollens allergy) [33–39]. Although highly specific, the problem

of this method is that it may only be applied to the patients exhibiting the HLA molecule for which the tetramer is available. Also, the repertoire of the T cells detected is by definition restricted to the peptides available in the tetramers. Finally, HLA class II tetramers needed to identify CD4⁺ Th2 cells are quite labour intensive to produce.

The present results also demonstrate that the number of HDM-specific IL-4- or IL-13-secreting T cells is significantly correlated with the severity of allergic rhinitis in the series of HDM asthmatic children studied. Thus, the level of the allergen-specific T cell response well reflected a clinically relevant HDM allergy. Indeed, the severity of allergic rhinitis could be inferred by the number of HDM-specific IL-4-secreting T cells. Interestingly, we found that this result was not influenced by the asthma control or the level of serum HDM-specific IgE. To our knowledge, this is the first time that a biological test is associated with a clinically symptomatic allergy independently of an exogenous (biological) sensitization. As the analyses were performed on circulating T cells, the present results also support the notion that, if adequately measured, the functional capacity of lymphoid T cells in the periphery is a good reflect of ongoing processes *in situ*, namely the immune-mediated allergic inflammatory process in the airway mucosa. This is relevant as no such relationship has been described for circulating versus local levels of specific IgE. Thus, in a series of patients presenting grass pollen allergy, specific IgE in the nasal mucosa showed a better correlation with allergen exposure than serum evaluations [40]. Similarly, in HDM-allergic children presenting rhinitis and asthma, specific IgE in the nasal mucosa increased significantly during allergen exposure, while serum HDM-specific IgE did not [41].

Further confirming the specificity of the quantification of circulating HDM-specific IL-4- or IL-13-secreting T cells for identifying the inflammatory allergic process and its severity is the finding that these results are seen in all seasons. In fact, as we shall see below, although the number of HDM-specific T cells fluctuates with the season, results still correlate with the severity of the rhinitis.

Despite the fact that HDM allergy is a well-known per annual allergy, it was described in different studies and in different geographical regions that HDM allergen levels were influenced by seasons. In Australia, Derp 1 levels were measured in beds and peaked during late autumn with a two- to threefold higher concentration than in summer. Allergen peaks also correlated with relative humidity. Seasonal changes in allergen were not affected by the HDM avoidance intervention [42]. Similar results were found in Canada [43]. In the same vein, cold winter decreased Der p1 concentration in German mattress [44]. Reflecting these seasonal varia-

tions, biological parameters were also reported in the literature to fluctuate during the year. In Korea, Derf 1, a major allergen of the most frequent mite in this area, *dermatophagoides farinae*, were found increased in bedding in August and September compared with November and December, and specific IgE and IgG4 to Derf 1 showed same trends [45, 46]. In the Netherlands, seasonal changes were seen in serum total IgE and number of peripheral blood eosinophil corresponding to an increase in Derp 1 concentration in floor dust in autumn [47].

Our present results show that the number of circulating HDM-specific IL-4- or IL-13-secreting T cells is increased in Autumn and early spring which are two wet seasons with high concentrations in HDM allergens as described in the literature. This suggests that an increase in HDM allergens in the environment could be the triggering factor for the increased number of HDM-specific IL-4- or IL-13-secreting T cells. These results are in keeping with the recent report showing a significant increase in the number of HDM-specific IL-13- and IL-4-secreting T cells observed 5 weeks after a bronchial provocation test to HDM [28].

In conclusion, if confirmed on a larger series of patients our data point to the potential interest of the ELISpot assay, we described in the characterization of allergen-specific T cells for an improvement of diagnosis and monitoring. In addition, this tool may also benefit for a better selection of patients enrolled in desensitization studies.

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Author contributions

B. M, L. C, J. J: designed the research, interpreted the data and wrote the manuscript; B. M: performed the experiments, collected and analysed the data; J. J: coordinated the recruitment of the patients; R. G-E: contributed to the recruitment of the patients; S. C, R. C: contributed analytical tools; J. P. Jais: contributed to the statistical analysis; J-F. B: supervised the research and provided critical revision of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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