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Correlation of Der p 2 T-cell responses with clinical characteristics of children allergic to house dust mite

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ABSTRACT

Background: An understanding of the mechanisms responsible for the development and maintenance of allergic inflammation and their clinical implications is needed to develop specific and successful treatment for allergy.

Objectives: To characterize in vitro T-cell responses to Der p 2, one of the major allergens of house dust mite (HDM), and investigate potential correlations between clinical and laboratory parameters.

Methods: Forty-two patients monosensitized to HDM and 10 age-matched, healthy children were studied. Dendritic cells pulsed with Der p 2 were used to stimulate autologous CD14⁺ cells. Der p 2-specific T-cell activation markers, proliferation, and cytokine production profiles were examined.

Results: Der p 2-specific T-cell activation markers, proliferation, and T_H2 cytokine production were significantly higher in HDM patients compared with healthy controls. Moreover, a significant correlation between proliferation and T_H2 cytokine production was observed. Within the allergic group, skin reaction to HDM was significantly stronger in patients with a Der p 2-specific T-cell response. Levels of HDM-specific IgE directly correlated with interleukin 5 and interleukin 13 levels and with skin prick test results and, ultimately, with the patient's family history of allergy. Furthermore, the presence of atopic march correlated with T-cell proliferation.

Conclusions: We found that, in HDM patients, Der p 2-specific T_H2 responses, promoted by autologous dendritic cells in vitro, correlate with clinical parameters.

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Introduction

Allergy to house dust mites (HDMs) of the *Dermatophagoides* species, *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*, is one of the most relevant health problem in childhood, affecting approximately 15% of the individuals in Western countries. Several allergens are involved in the immune responses to HDM, although group 1 (Der f 1 and Der p 1) and group 2 (Der f 2 and Der p 2) allergens are considered the major HDM allergens in terms of clinical symptoms. More than 97% of HDM-sensitive individuals are sensitized to the major Der p 1 and Der p 2 allergens,¹ but it is well known that

there is an important cross-reaction between Der p and Der f allergens because human sensitivity to both mites is frequently observed.²

The hallmark of the immune reactivity to allergens is a T_H2 skewed response that causes a cascade of cellular events (eg, eosinophilia, B-cell isotype switching to IgE, and mast cell degranulation) in target tissues. Moreover, recently it has emerged that some HDM allergens, due to their constitutive characteristics, can induce a broader spectrum of immune responses, including the triggering of the innate immune system. For instance, the role of Der p 2 in promoting modifications of structural airway cells has been recently highlighted.^{3,4} Indeed, through interaction with Toll-like receptor 2 and Toll-like receptor 4, Der p 2 activates nuclear factor-κB and mitogen-activated protein kinases and promotes the secretion of proinflammatory cytokines in airway smooth muscle cells and epithelial cells.^{4–6} Notably, airway smooth muscle cells cultured with Der p 2 displayed increased ERK1/2 phosphorylation and c-Fos expression, important factors involved in promoting T_H2 immune responses.⁵ These findings indicate that, in addition to their immunogenic properties, nonproteolytic allergens, such as

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Der p 2, can exacerbate respiratory airway diseases by activating respiratory epithelial cells.

As for other forms of allergy, therapeutic options for HDM-related allergic diseases are limited to symptom-controlling drugs, even though allergen specific immunotherapy has gained wide acceptance.^{7,8} However, despite the many efforts to improve allergen specific immunotherapy efficacy and safety, there is still a need to characterize the immunologic mechanism specific for each allergen and to identify biomarkers that may predict the clinical response to the treatment.

Recently, we demonstrated that autologous monocyte-derived dendritic cells (DCs) pulsed with Der p 2 (Dp2-DCs) are efficient in promoting Der p 2–specific T_H2 response in T cells from HDM-allergic patient but not in healthy controls.⁹ Thus, in the present study, in a cohort of children allergic to HDM, we evaluated the in vitro responses of T cells stimulated by Dp2-DCs with the aim of investigating potential correlations between clinical and immunologic parameters.

Methods

Study population

The study included 42 allergic children (18 girls and 24 boys; mean [SD] age, 102.8 [39.6] months; age range, 36–172 months) with a history of mild allergic symptoms (eg, asthma, rhinoconjunctivitis, and atopic dermatitis) after HDM exposure. The diagnosis of HDM allergy was determined by positive skin prick test (SPT) results to Der p and Der f extracts (Stallergenes, Milan, Italy; extract titer, 100 IR/mL; Der p 2 concentration, 4 µg/mL) and/or an elevated specific IgE level (CAP/FEIA; Phadia & Upjohn, Uppsala, Sweden). A positive SPT result was defined as a wheal 3 mm greater in diameter than the negative control reaction. Patients tested negative for cow milk, egg, grass, birch, *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium*, *Olea* tree, and cat and dog dander. Severity of allergic symptoms was graded according to the classification of the Global Initiative for Asthma,¹⁰ Allergic Rhinitis and its Impact on Asthma,¹¹ and SCORAD (SCORing Atopic Dermatitis) Index for dermatitis.¹² Twenty of 42 patients had a history of atopic march, defined as the clinical evolution and

progression of allergic symptoms from cutaneous to respiratory manifestations.¹³ At the time of enrollment all children underwent a 2-week steroid washout. The demographic and clinical characteristics of the patients are reported in Table 1. Ten age-matched, healthy children (5 boys and 5 girls; mean [SD] age, 140.4 [49.2] months; age range, 42.0–207.6 months), without a personal and familial history of allergy, were used as controls to evaluate the specificity of the response of the allergic children. The study was approved by the local medical ethics committee (Policlinico di Tor Vergata, Tor Vergata University, Rome, Italy), and informed oral consent was obtained from parents.

Cells preparation

Peripheral blood mononuclear cells (PBMCs) were separated from peripheral venous blood of patients and controls by Ficoll-Hypaque density gradient centrifugation (Amersham Biosciences, Uppsala, Sweden).

Dendritic cell differentiation

CD14⁺ monocytes were isolated from PBMCs by positive selection using anti-CD14–conjugated magnetic Microbead (MACS; Miltenyi Biotec, Belgisch Gladbach, Germany) according to the manufacturer's instruction. The negative fraction (CD14[−]), mostly constituted by T cells (CD3⁺ routinely >85%, as checked by flow cytometry), was collected and cryopreserved for later use.

DCs were differentiated from CD14⁺ monocytes and left unstimulated or cultured with extractive purified endotoxin-free Der p 2 (1 µg/mL; Lofarma, Milan, Italy) (Dp2-DCs) for 2 days, as previously described.⁹ Purity and maturation state were routinely checked by flow cytometric analysis by determination of CD14, CD1a, CD80, CD83, CD86, and HLA-DR expressions.⁹

Der p 2–specific T-cell proliferative response

Autologous CD14[−] cells (5×10^4 per well) were thawed and cultured with immature DC (iDCs) and Dp2-DCs (5×10^3 per well) (CD14[−] cell to DC ratio of 10:1) in 96-well, round-bottom microplates in triplicate in a final volume of 200 µL of medium. After 5 days of culture, proliferation was assessed by overnight [³H]thymidine incorporation (1 µCi per well; Amersham International, Amersham, United Kingdom). Before [³H]thymidine incorporation, 50 µL of supernatant from each well was collected for cytokine analysis. The value of cell proliferation was normalized to the one observed after stimulation by iDCs (stimulation index [SI]). Responder was defined as any patient with an SI greater than or equal to the mean SI plus 2 SDs obtained by stimulating CD14[−] cells of healthy controls. On this basis the cutoff was set at an SI of 1.4.

To test the specific response to Der p 2, DCs of 3 patients were stimulated with extract of cow's milk protein mix, α-lactalbumin, β-lactoglobulin, and α-casein (10 µg/mL each; Sigma-Aldrich, St Louis, Missouri) during the 2 last days of culture (CM-DCs). Cow's milk proteins were previously tested for endotoxin content by the LAL test (Cambrex Bio Science, Walkersville, Maryland) and resulted in less than 10 EU/mL. Autologous CD14[−] cells (5×10^4 per well) were cultured with iDCs, Dp2-DCs, and CM-DCs (5×10^3 per well) at a 10:1 T cell/DC ratio in 96-well, round-bottom microplates in triplicate in a final volume of 200 µL of medium. After 5 days of culture, CD14[−] cell proliferation was assessed as described.

T-cell characterization

CD14[−] cells were stained before and after 5 days of culture with Dp2-DCs with monoclonal antibodies against surface phycoerythrin (PE)–cyanine 5 (Cy5)–CD3, fluorescein isothiocyanate (FITC)–CD4, FITC–CD8, PE–CD25, and intracellular PE–cytotoxic T-lymphocyte antigen 4 (CTLA-4), as well as isotype controls,

Table 1
Characteristics of the study cohort

Characteristic	Finding (n=42)
Sex, F/M, No.	18/24
Age, mean (SD), mo	102.8 (39.6)
Family history, No. (%)	
Allergy	30/42 (71.4)
Asthma	7/30 (23.3)
Rhinoconjunctivitis	6/30 (20.0)
Atopic dermatitis	3/30 (10.0)
Family history of autoimmunity	8/42 (19.0)
Main symptom of allergy, No. (%)	
Asthma	16/42 (38.1)
Rhinoconjunctivitis	19/42 (45.2)
Atopic dermatitis	7/42 (16.7)
Atopic march ^a	20/42 (47.6)
Symptoms duration, mean (SD), mo	45.4 (32.3)
Respiratory infections during the follow-up, No. (%)	3/42 (7.1)
Total IgE, mean (SD), IU/mL	169.9 (208.2)
Specific IgE for Der p, mean (SD), kUA/mL ^b	21.6 (33.6)
Specific IgE for Der f, mean (SD), kUA/mL ^b	12.2 (19)
Skin prick test for Der p, mean (SD), mm ^c	6.3 (1.5)
Skin prick test for Der f, mean (SD), mm ^c	6.5 (1.7)
Therapy, No. (%) ^d	39/42 (86.7)

^aAtopic march was defined as the clinical evolution and progression of allergic symptoms from cutaneous to respiratory manifestations.

^bSpecific IgE were considered positive if 0.35 kUA/mL or higher.

^cPositive skin prick test result was defined as a wheal 3 mm greater in diameter than the negative control reaction.

^dOral antihistamine treatment administered only in patients with active symptoms.

FITC- or PE-, Cy5-, and APC-conjugated (all from BD Biosciences, San Jose, California). For intracellular expression of CTLA-4, after surface staining, a Cytoperm/Cytofix kit (BD Pharmingen, San Diego, California) was used, according to the manufacturer's instructions. After washing twice, cells were incubated with anti-CTLA-4 for 20 minutes, washed, and analyzed by multiparametric flow cytometry with FACSCalibur (Becton Dickinson), interfaced with Cellquest software (BD Biosciences).

Cytokine production assay

The levels of interleukin (IL) 4, IL-5, IL-10, IL-13, and interferon γ (IFN- γ) were determined in CD14⁺-DC culture supernatants after 5 days of culture with the Bioplex Protein Array System according to the manufacturer's instruction (BioRad, Hercules, California).

Statistical analysis

To verify the distribution of samples, a 1-sample Kolmogorov-Smirnov test was used. Results are presented as mean (SD) or mean (SEM) (only for cytokines production analysis), unless differently stated. The *t* test was used to compare the means of cell proliferation and cytokine production between patients and controls and among different cell culture conditions and to compare SI values between patients with or without atopic march. When the values were nonparametric, we used the Mann-Whitney test. Association among the variables was assessed using the parametric Pearson correlation test or the nonparametric Spearman correlation test as appropriate. $P < .05$ was considered statistically significant.

Results

Der p 2-pulsed DCs and a Der p 2-specific T-cell response

A significant increase of CD25⁺CD4⁺ T-cell frequency was observed after culture with Dp2-DCs (11.3% [5.2%] vs 29.9% [13.1%] in CD4⁺ T cells before and after coculture, respectively; $n=14$;

$P < .001$). Moreover, we observed that the percentage of CTLA-4⁺CD4⁺ T cells after 5 days of coculture was significantly higher compared with time 0 (2.3% [0.9%] vs 26.0% [11.3%] at time 0 and time 5, respectively; $n=11$; $P < .001$) (Figure 1A). Notably, CD25 expression correlated with total IgE production ($n=13$, $P=.02$, data not shown).

The proliferative response of CD14⁺ cells cultured with Dp2-DCs expressed as the SI was significantly higher in HDM patients ($n=42$) than in the control group ($n=10$) (3.0 [2.9] vs 1.0 [0.2], $P < .001$). Among allergic children, Der p 2-specific proliferation was observed in 33 of 42 patients (78.6% responders).

To evaluate allergen specificity, CD14⁺ cells from 3 Der p 2 responders were tested in parallel for proliferation with autologous CM-DCs, and no response was observed (0.8 [0.2] vs 3.3 [1.3] CMP-DCs vs Dp2-DCs, respectively; $P=.04$).

Der p 2-pulsed DCs and a T_H2 Cell response in HDM patients

We then evaluated the ability of CD14⁺ cells cultured with Dp2-DCs to secrete IL-5 and IL-13. CD14⁺ cells stimulated with Dp2-DCs from 28 HDM patients ($n=24$ Der p 2 responders and 4 Der p 2 nonresponders) secreted significantly higher amounts of IL-5 (294.1 [385.6] pg/mL, $n=28$) compared with cells activated with iDCs (31.8 [77.6] pg/mL, $P=.005$) (Figure 1B). Similarly, CD14⁺ cells cultured with Dp2-DCs from 22 HDM patients ($n=18$ Der p 2 responders and 4 Der p 2 nonresponders) secreted higher levels of IL-13 (165.3 [311.0] pg/mL, $n=22$) compared with CD14⁺ cells activated with iDCs (6.9 [14.2] pg/mL) (Figure 1C).

CD14⁺ cells from 26 patients stimulated with Dp2-DCs secreted IFN- γ at similar levels to those produced by CD14⁺ cells primed with iDCs (83.0 [165.4] pg/mL vs 80.1 [163.4] pg/mL, respectively; $n=26$; Figure 1D). Notably, nonresponder patients ($n=4$), compared with responder patients ($n=24$ and 18), produced small amounts of IL-5 and IL-13 despite a conserved production of IFN- γ (Fig 1A-C).

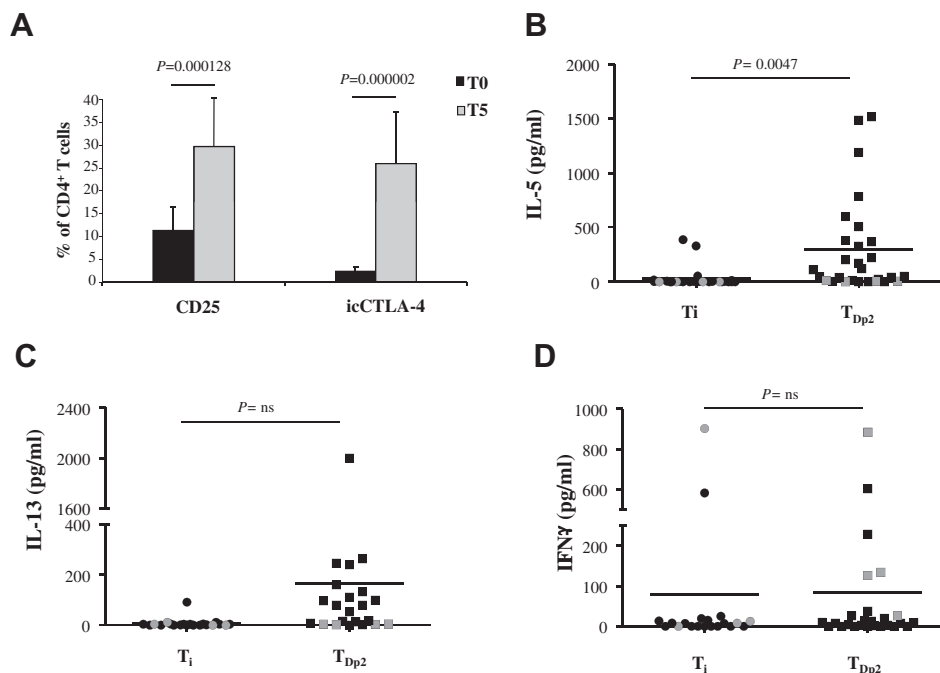


Figure 1. Der p 2 pulsed dendritic cells (DCs) promote T-cell activation and T_H2 cell response in house dust mite (HDM) patients. CD14⁺ cells from HDM allergic patients were stimulated with autologous immature DC (T_i) and Der p 2 DCs (T_{Dp2}) at a 10:1 ratio for 5 days. Culture supernatants were collected after 5 days of coculture, and cytokine concentration was determined with the Bioplex Protein Array system. Surface expression of CD25 and intracellular expression of cytotoxic T-lymphocyte antigen 4 (icCTLA-4) on CD4⁺ T cells were evaluated by FACS analysis at day 0 (T₀) and after 5 days of coculture (T₅). (A) Histograms represent the mean ($\pm$ SD) percentage ($n=14$). Production of interleukin (IL) 5 ($n=28$) (B), IL-13 ($n=22$) (C), and interferon γ (IFN- γ) (D) by T_i and T_{Dp2} cells. Gray dots indicate nonresponder patients, whereas black dots indicate responder patients. Responder was defined as any patient displaying a stimulation index of $\geq$ mean SD plus 2 SDs obtained by stimulating T cells of healthy controls with autologous Dp2-DCs.

We also tested the production of IL-10, and, as expected, we found that CD14⁺ cells stimulated with autologous Dp2-DCs produced small amounts of IL-10 (4.6 [4.7] pg/mL, n=25).

Correlation of clinical and immunologic parameters

Within the cohort of HDM patients, we evaluated possible correlations between clinical and immunologic parameters. As expected, in all patients, IL-5 production significantly correlated with IL-13 production ($P<.001$). Moreover, we observed a significant correlation between the production of both T_H2 cytokines, IL-5 and IL-13, and SI ($P=.001$, n=25, and $P=.003$, n=20, respectively) (Fig 2A and B).

As shown in Figure 3A and B, specific IgE and SPT to the allergen Der p correlated with the levels of IL-5 ($P=.001$ and $.001$, respectively; n=22) and IL-13 produced by CD14⁺ cells stimulated with Dp2-DCs ($P=.03$ and $.05$, respectively; n=22). In all patients, Der p-specific IgE correlated with the corresponding SPT results ($P<.001$, n=42). Furthermore, the size of skin reaction to HDM, as evaluated by SPT, was significantly greater in responder vs non-responder patients ($P=.045$) (Fig 4A). Interestingly, in all patients, Der p-specific IgE correlated with the patients' family history of allergy ($P=.046$, data not shown) and proliferation of CD14⁺ cells stimulated with Dp2-DCs with the presence of atopic march ($P=.01$) (Fig 4B). No other correlations between the clinical and the immunologic parameters evaluated were found.

Discussion

We report the in vitro T-cell response to autologous DCs pulsed with Der p 2, one of the major allergens of HDM, in a cohort of allergic children monosensitized to HDM. This group of patients

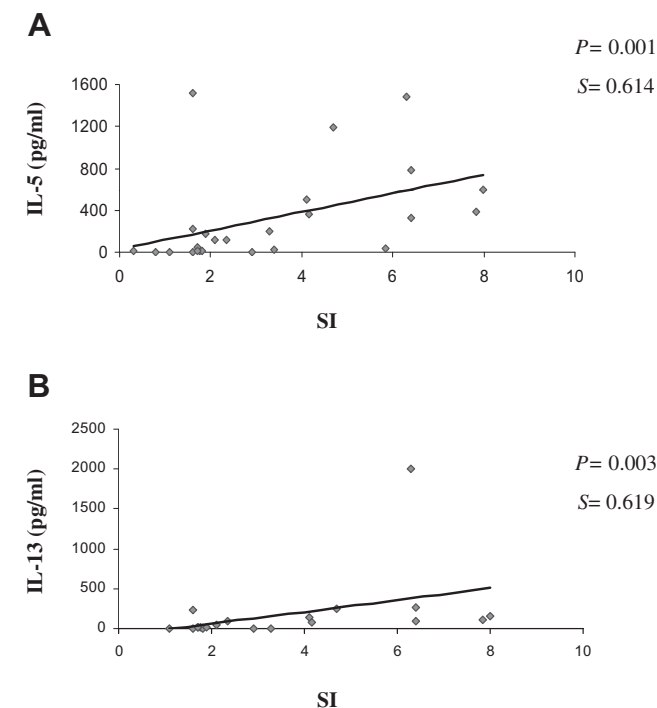


Figure 2. T_H2 cytokine production (interleukin [IL] 5 and IL-13) correlates with T-cell proliferation to Der p 2. CD14⁺ cells from house dust mite allergic patients (n=42) were stimulated with autologous immature dendritic cells and Der p 2 dendritic cells at a 10:1 ratio for 5 days. Proliferative responses were evaluated after 5 days of culture by overnight [³H]thymidine incorporation. Results are expressed as the stimulation index (SI). Culture supernatants were collected after 5 days of coculture, and cytokine concentration was determined by the Bioplex Protein Array system. Each dot refers to an individual patient. A, IL-5 (n=25); B, IL-13 (n=20).

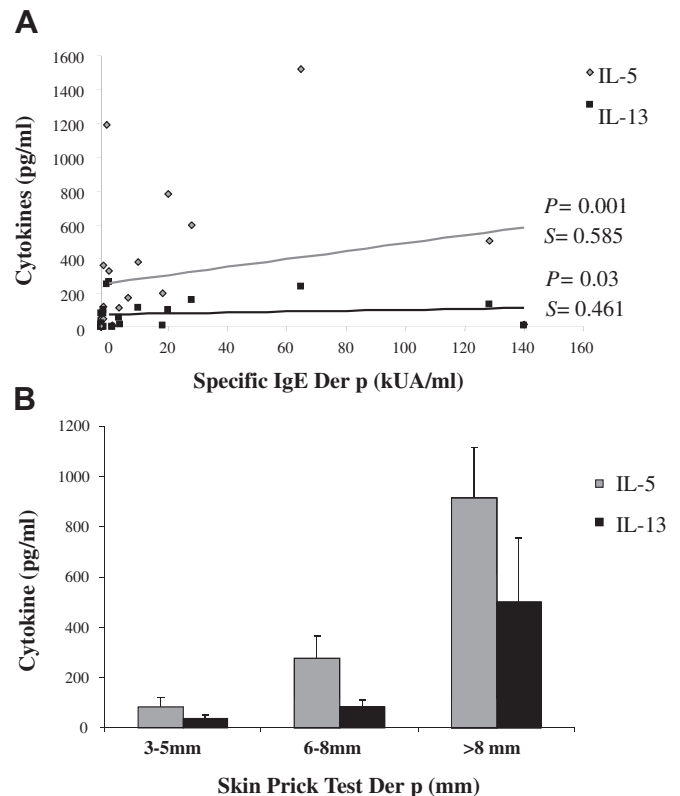


Figure 3. T_H2 cytokine production correlates with allergologic parameters. CD14⁺ cells from house dust mite allergic patients (n=42) were stimulated with autologous immature dendritic cells and Der p 2 dendritic cells at a 10:1 ratio for 5 days. Culture supernatants were collected after 5 days of coculture, and cytokine concentration was determined by the Bioplex Protein Array system. A, Der p specific IgE correlation with interleukin (IL) 5 and IL-13 production. Each dot refers to an individual patient (n=22). B, Correlation of Der p skin prick test with IL-5 and IL-13 production (n=22). Histograms represent the mean (+SEM). Statistical analysis was performed by the Spearman correlation test.

belongs to a larger cohort already investigated with the aim of demonstrating that it is possible to down-modulate T-cell response to Der p 2 through the manipulation of DCs.⁹

In the present study, first we confirmed and expanded the notion that stimulation of T cells with DCs pulsed with CD14⁺ is a powerful tool to activate T cells and induces a Der p 2-specific T-cell proliferation and a T_H2 cytokine production (IL-5 and IL-13). Second, but more important, the study led to the identification of several correlations between immunologic and clinical parameters that might have important clinical and therapeutic implications.

Concerning the characterization of T cells, in our cohort of HDM-allergic children, activation of T cells with Dp2-DCs resulted in the upregulation of CD25 and CTLA-4 on CD4⁺ T cells. Sicherer et al¹⁴ recently reported that after in vitro stimulation with milk or egg allergen, 70% to 80% of mononuclear cells express CD3, CD4, and CD25. CD25 is the receptor of IL-2, and it has been established that the IL-2 pathway has a crucial role in the homeostasis of T-regulatory cells and therefore in the tolerance to allergens. In murine models of asthma and allergy, it has been found that the activation of the CTLA-4 pathway can prevent or block airway hyperreactivity and allergic immune response.^{15,16} Thus, it can be speculated that the upregulation of CD25 and CTLA-4 induced in CD4⁺ T cells by Dp2-DCs in vitro is required in vivo for dampening T-cell response to HDM.

Using Der p2-DCs, we demonstrated that T cells from HDM children, but not from healthy controls, proliferate specifically to Der p 2. Within the atopic cohort, 78.6% of the patients responded

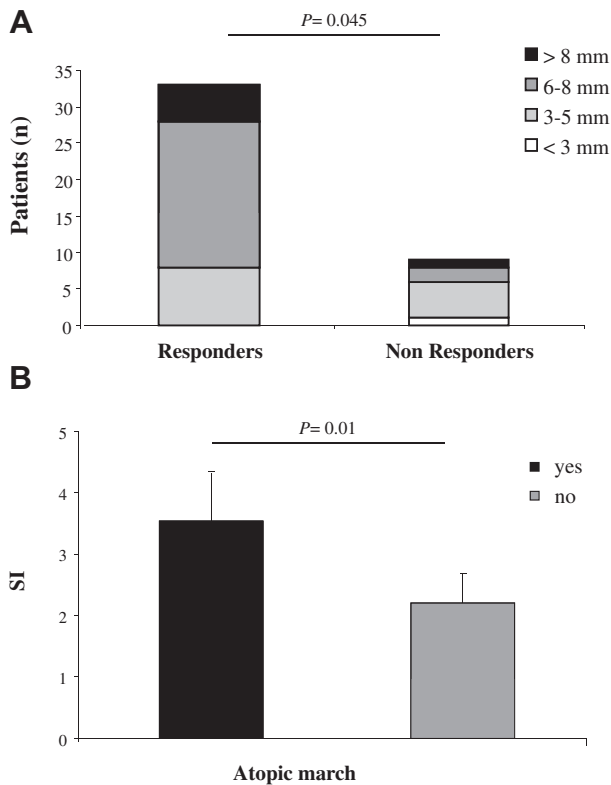


Figure 4. Correlation between T-cell response and allergologic and clinical phenotype. CD14⁺ cells from house dust mite allergic patients (n=42) were stimulated with autologous immature dendritic cells and Der p 2 dendritic cells at a 10:1 ratio for 5 days. Proliferative responses were evaluated after 5 days of culture by overnight [³H]thymidine incorporation. Responder was defined as any patient displaying a stimulation index of a mean SI plus 2 SDs obtained by stimulating CD14⁺ of healthy controls with autologous Der p 2 dendritic cells. A, The distribution of the skin prick test (SPT) dimension in responder (n=33) and nonresponder (n=9) patients is shown. SPTs were performed as described in the "Methods" section. B, Atopic march was defined as the presence of the sequential occurrence of allergic manifestations during early childhood. The histograms represent the mean (±SD) of the stimulation index in patients with (n=13) or without (n=21) atopic march.

to Der p 2, consistent with previous reports in which 80% to 90% of mite-sensitive individuals have IgE antibodies to Der p 1 and 2 allergens.^{1,17} The reported high frequency of Der p 2 reactive patients is probably due to the presence of an immunodominant T-cell epitope in Der p 2 that behaves like a universal epitope recognized by most human major histocompatibility class II molecules.¹⁸

Furthermore, we found that Dp2-DCs, in most of our cohort, induced a T_H2 cytokine profile. The ability of allergen-pulsed DCs to stimulate a T_H2 cell response in an autologous system has been previously demonstrated. For instance, autologous Der p 1-pulsed DCs have been found to be able to stimulate T cells from allergic patients and to preferentially increase IL-4 production without modifying IFN- γ production. Conversely, autologous Der p 1-pulsed DCs favored a type 1 response in healthy donors.¹⁹ Our results on Der p 2 are substantially in line with several previous reports in which stimulation of total PBMCs from HDM allergic patients with Der p 2 promoted T-cell proliferation and T_H2 cytokine induction^{20,21} and with the work of Sung et al,²² who demonstrated that DCs derived from adult allergic patients, stimulated with Der p 2, are able to elicit a strong T_H2 response.

An increase of the T_H1 response has been also reported in chronic allergic inflammation,²³ and IFN- γ production by T cells stimulated with allergen was observed.^{22,24} In our patient we also

detected IFN- γ production, but no correlations were observed between the amounts of IFN- γ and other immunologic or clinical parameters in our cohort; therefore, we would not envisage this cytokine as a valuable clinical marker.

We then investigated IL-10 and found that it was poorly produced by our patients. Although in an animal model of atopic eczema it has been demonstrated that IL-10 produced by antigen-presenting cells and T cells plays a key role in T_H2 cell development,²⁵ the extensive research performed in the last years on IL-10 demonstrated a mainly regulatory role for this cytokine in allergy.^{23,26-29} Our results are in line with this concept.

In addition to the immunologic findings, several key, clinically relevant observations were made. With respect to the correlation between the tests performed in vivo with Der p whole extract and the tests performed in vitro with purified Der p 2, the difference of the reagents might raise some concern. Nevertheless, it is known that all Der p extracts contain Der p 1 and Der p 2 and that the contribution of minor allergens to the overall reactivity of mite allergic patients to the commercial diagnostic tests is minimal. Therefore, we considered the reactivity of our patients to commercial standardized extracts of Der p as expression of reactivity to major antigens.

It is known that atopic infants have an earlier and steeper increase in serum IgE levels during their early years of life compared with nonatopic controls.³⁰ Moreover, it has recently been demonstrated that Der p 1-specific IgE concentrations are significantly higher in children with asthma sensitized to HDM with respect to healthy controls and the levels tend to increase over time.³¹ In our patients, we did not observe a significant correlation between Der p-specific IgE values and age or disease duration. Nevertheless, we found that the Der p-specific IgE levels significantly correlate with the size of wheal and flare after SPT. Both parameters correlated with IL-5 and IL-13 but not with IFN- γ in vitro production, extending the notion that both of these cytokines are specifically important in the pathogenesis of allergic disease.^{25,32}

Our findings demonstrate that patients whose T cells responded in vitro to Dp2-DCs had the greatest skin reaction to HDM extract. The correlation between SPT and T-cell proliferation to allergens was rarely observed, and most of the studies reached the conclusion that skin reactivity is not a good indicator of T-cell response in allergic patients.³³ Besides the different nature of the allergen, this discrepancy should also be ascribed to the different method used; allergen responsiveness can be better detected with DCs pulsed with the allergen than with PBMCs.²² Although only Der p 2-specific responses were evaluated in this study, our results suggest that SPT is a reliable indicator for the intensity of T-cell responses against the specific allergen and might allow the identification of Der p 2-allergic patients who do not display detectable specific IgE in the serum.

Moreover, we identified a correlation between serum levels of specific IgE to Der p and patients' family history of allergy. If parental history can be considered an indicator of inherited susceptibility, as suggested in previous reports,³⁴ our study supports the notion that potential gene-environment interactions contribute to the etiology of sensitization and atopic manifestations.

Interestingly, we detected higher levels of T-cell proliferation in patients who underwent atopic march compared with patients who were clinically stable. This finding, to our knowledge, has never been reported and, once confirmed in a larger cohort of patients, might provide a useful marker to predict the evolution of symptoms in HDM allergic patients.

In conclusion, by using Dp2-DCs to promote T-cell responses to Der p 2, we demonstrated that the allergen specific immunologic events induced in vitro in T cells of HDM allergic patients correlate with some of their clinical features. The advances in the knowledge

of the role of DCs in allergic disease and the identification of predictive markers specific for clinical outcome could be helpful in the design of novel immunotherapies both in terms of disease targeting and prediction of successful responses to allergen specific immunotherapy.

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References

- [1] Weghofer M, Thomas WR, Kronqvist M, et al. Variability of IgE reactivity profiles among European mite allergic patients. *Eur J Clin Invest*. 2008;38:959–965.
- [2] Smith AM, Benjamin DC, Hozic N, et al. The molecular basis of antigenic cross-reactivity between the group 2 mite allergens. *J Allergy Clin Immunol*. 2001;107:977–984.
- [3] Lloyd CM. Dust mites' dirty dealings in the lung. *Nat Med*. 2009;15:366–367.
- [4] Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med*. 2009;15:410–416.
- [5] Chiou YL, Lin CY. Der p 2 activates airway smooth muscle cells in a TLR2/MyD88-dependent manner to induce an inflammatory response. *J Cell Physiol*. 2009;220:311–318.
- [6] Osterlund C, Grönlund H, Polovic N, Sundström S, Gafvelin G, Bucht A. The non-proteolytic house dust mite allergen Der p 2 induce NF-kappaB and MAPK dependent activation of bronchial epithelial cells. *Clin Exp Allergy*. 2009;39:1199–1208.
- [7] Canonica GW, Bousquet J, Casale T, et al. Sub-lingual immunotherapy: World Allergy Organization Position Paper 2009. *Allergy*. 2009;64:1–59.
- [8] Cox L, Esch RE, Corbett M, Hankin C, Nelson M, Plunkett G. Allergen immunotherapy practice in the United States: guidelines, measures, and outcomes. *Ann Allergy Asthma Immunol*. 2011;107:289–299.
- [9] Pacciani V, Gregori S, Chini L, et al. Induction of anergic allergen-specific suppressor T cells using tolerogenic dendritic cells derived from children with allergies to house dust mites. *J Allergy Clin Immunol*. 2010;125:727–736.
- [10] Bousquet J, Clark TJ, Hurd S, et al. GINA guidelines on asthma and beyond. *Allergy*. 2007;62:102–112.
- [11] Bousquet J, Khaltaev N, Cruz AA, et al. Allergic Rhinitis and its Impact on Asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(suppl 86):S8–S160.
- [12] Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol*. 1980;92:44–47.
- [13] Weinberg EG. The atopic march. *Curr Allergy Clin Immunol*. 2005;18:4–5.
- [14] Sicherer SH, Wood RA, Stablein D, et al. Immunologic features of infants with milk or egg allergy enrolled in an observational study (Consortium of Food Allergy Research) of food allergy. *J Allergy Clin Immunol*. 2010;125:1077–1083.
- [15] Deurloo DT, van Esch BC, Hofstra CL, Nijkamp FP, van Oosterhout AJ. CTLA4-IgG reverses asthma manifestations in a mild but not in a more "severe" ongoing murine model. *Am J Respir Cell Mol Biol*. 2001;25:751–760.
- [16] van Wijk F, Nierkens S, de Jong W, et al. The CD28/CTLA-4-B7 signaling pathway is involved in both allergic sensitization and tolerance induction to orally administered peanut proteins. *J Immunol*. 2007;178:6894–6900.
- [17] Taketomi EA, Silva DA, Sopelete MC, Gervásio AM, Alves R, Sung SJ. Differential IgE reactivity to Der p 1 and Der p 2 allergens of *Dermatophagoides pteronyssinus* in mite-sensitized patients. *J Invest Allergol Clin Immunol*. 2006;16:104–109.
- [18] Wu B, Elst LV, Carlier V, Jacquemin MG, Saint-Remy JM. The *Dermatophagoides pteronyssinus* group 2 allergen contains a universally immunogenic T cell epitope. *J Immunol*. 2002;169:2430–2435.
- [19] Hammad H, Charbonnier AS, Duez C, et al. Th2 polarization by Der p 1–pulsed monocyte-derived dendritic cells is due to the allergic status of the donors. *Blood*. 2001;98:1135–1141.
- [20] O'Brien RM, Thomas WR, Wootton AM. T cell responses to the purified major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol*. 1992;89:1021–1031.
- [21] Bullens DM, De Swertdt A, Dilissen E, et al. House dust mite-specific T cells in healthy non-atopic children. *Clin Exp Allergy*. 2005;35:1535–1541.
- [22] Sung SJ, Taketomi EA, Smith AM, Platts-Mills TA, Fu SM. Efficient presentation of house dust mite allergen Der p 2 by monocyte-derived dendritic cells and the role of beta 2 integrins. *Scand J Immunol*. 1999;49:96–105.
- [23] Akdis M, Verhagen J, Taylor A, et al. Immune responses in healthy and allergic individuals are characterized by a fine balance between allergen-specific T regulatory 1 and T helper 2 cells. *J Exp Med*. 2004;199:1567–1575.
- [24] Heaton T, Rowe J, Turner S, et al. An immunoepidemiological approach to asthma: identification of in-vitro T-cell response patterns associated with different wheezing phenotypes in children. *Lancet*. 2005;365:142–149.
- [25] Laouini D, Alenius H, Bryce P, Oettgen H, Tsimikou E, Geha RS. IL-10 is critical for Th2 responses in a murine model of allergic dermatitis. *J Clin Invest*. 2003;112:1058–1066.
- [26] Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol*. 2005;5:271–283.
- [27] Bohle B, Kinaciyan T, Gerstmayr M, Radakovic A, Jahn-Schmid B, Ebner C. Sublingual immunotherapy induces IL-10-producing T regulatory cells, allergen-specific T-cell tolerance, and immune deviation. *J Allergy Clin Immunol*. 2007;120:707–713.
- [28] Meiler F, Zumkehr J, Klunker S, Rückert B, Akdis CA, Akdis M. In vivo switch to IL-10-secreting T regulatory cells in high dose allergen exposure. *J Exp Med*. 2008;205:2887–2898.
- [29] Angelini F, Pacciani V, Corrente S, et al. Dendritic cells modification during sublingual immunotherapy in children with allergic symptoms to house dust mites. *World J Pediatr*. 2011;7:24–30.
- [30] Barbee RA, Brown WG, Kaltenborn W, Halonen M. Allergen skin-test reactivity in a community population sample: correlation with age, histamine skin reactions and total serum immunoglobulin E. *J Allergy Clin Immunol*. 1981;68:15–19.
- [31] Aydogan M, Mete N, Yazici D, et al. Comparison of Der p1-specific antibody levels in children with allergic airway disease and healthy controls. *Pediatr Allergy Immunol*. 2007;18:320–325.
- [32] Weber-Chrysochoou C, Crisafulli D, Almqvist C, et al. for the Childhood Asthma Prevention Study Investigators. IL-5 T-cell responses to house dust mite are associated with the development of allergen-specific IgE responses and asthma in the first 5 years of life. *J Allergy Clin Immunol*. 2007;120:286–292.
- [33] Benítez D, García-Ortega P, Picado C, et al. Specific immune response to *Parietaria judaica* plant profilin: a low T cell proliferative response supports high IgE and skin prick test. *Allergol Immunopathol (Madr)*. 2002;30:62–69.
- [34] Alford SH, Zoratti E, Peterson EL, Maliarik M, Ownby DR, Johnson CC. Parental history of atopic disease: disease pattern and risk of pediatric atopy in offspring. *J Allergy Clin Immunol*. 2004;114:1046–1050.