

Specific immune response to *Parietaria judaica* plant profilin: a low T cell proliferative response supports high IgE and skin prick test

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SUMMARY

Background: allergic disease caused by *Parietaria judaica* (Pj) has been widely documented in Mediterranean area. Profilins have been identified as widely distributed allergenic proteins. The role of Pj profilin in specific immune response in Pj-sensitized patients is unknown.

Methods: skin prick test and determination of specific and total IgE levels in serum were performed in all patients (n = 28) and non-allergic controls (n = 18). Peripheral blood mononuclear cells (PBMC) were isolated from both groups and stimulated with crude extract or highly purified Pj profilin. The production of type I and type II cytokines was determined by specific and polyclonal stimuli in patients and controls. T-cell lines specific to Pj profilin were established and cross-reactivity with another highly purified profilin from *Phleum pratense* (Phl p) was evaluated.

Results: Pj profilin-sensitized patients showed a small but significantly increased in T-cell proliferative response to this profilin compared with non-atopic controls. The production of interleukin (IL)-4 and interferon (IFN)- γ in response to the specific stimulus was undetectable. However, the production of IL-4 in response to a polyclonal stimulus [phytohemagglutinin (PHA)] was significantly higher in atopic patients than in controls. The T-cell response did not correlate with the magnitude of response to skin prick tests with Pj profilin or with Pj-specific serum IgE levels. In addition, the production of IL-4 in response to a polyclonal

stimulus (PHA) did not correlate with the individual skin prick tests to Pj profilin or with Pj-specific IgE levels in serum. The T-cell lines tested showed no cross-reactivity with Phl p profilin.

Conclusions: our results suggest that Pj profilin is partly responsible for the T-cell-mediated response in patients allergic to Pj. The high skin reactivity to Pj profilin in these patients was accompanied by a small increase in the T-cell response to this profilin. The response was highly specific since Pj profilin specific T-cell lines showed no cross-reactivity with a highly homologous profilin from Phl p. The lack of correlation between the proliferative T-cell response and polyclonal IL-4 production with allergen-specific serum IgE and skin reactivity probably indicates that some of the responding T-cells may be involved in immune reactions other than those supporting IgE production.

Key words: T-cells. Profilins. Skin Prick test. Specific IgE. IL-4. IFN- γ . *Parietaria judaica*.

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Abbreviations used

SPT: Skin prick test
PBMC: Peripheral blood mononuclear cells
PBS: Phosphate-buffered saline
PHA: Phytohemagglutinin
TT: Tetanus toxoid
SI: Stimulation index
Pj: *Parietaria judaica*

Phl p: *Phleum pratense*

INTRODUCTION

Pollen from *Parietaria judaica* (Pj) is a major source of airborne allergens in the Mediterranean area, giving rise to seasonal allergies with clinical manifestations such as rhinitis, conjunctivitis and asthma. Profilins constitute a ubiquitous family of proteins that control actin polymerization in eukaryotic cells (1) and have been identified as allergens in several species of tree, grass, and weed pollens, and in some fruits and vegetables. Profilins are described as pan-allergens, because they are recognized by 20 % of all pollen-allergic patients (2-4), and have been considered responsible for combined allergies to pollen and food (4).

It is well established that allergen-specific T_{H2}-like cells play an important role in the induction of IgE synthesis (IL-4) and eosinophilia (IL-5) (5-7). Furthermore, a higher frequency of infiltrating T-cells producing T_{H1} cytokines (IFN- γ) (8-10) and an increase in allergen-specific IgG (11) are found after successful immunotherapy. This stresses the importance of different subsets of T-cells in the allergic immune responsiveness. Whereas skin prick test (SPT) areas and allergen-specific IgE measurements are well correlated (12, 13), no relationship between allergen-specific T-cell proliferation, cytokine production, allergen-specific IgE, and SPT, has been found (14).

In this study we examine the specific immune response to Pj plant profilin in atopic patients and non-atopic subjects. We analyze the possible correlation between allergen-specific T-cell proliferation, cytokine production, allergen-specific IgE and SPT in atopic patients to Pj profilin and non-atopic donors. In addition, we address the question of the cross-reactivity between Pj and Phl p profilins.

METHODS

Characterization of blood donors

Blood samples from 28 Pj profilin allergic patients (mean age 32 years, range 19-54) and 18 non-atopic subjects (mean age 39 years, range 25-53) were collected. All patients had a clinical history of seasonal allergic rhinitis and/or asthma, positive SPT (> 4 mm of wheal diameter) to Pj pollen extracts and Pj profilin, and all but one had positive serum Pj-specific IgE (CAP-System, Pharmacia, Uppsala,

Sweden) levels in serum. None of the patients had received specific immunotherapy. Histamine chloxyde, 10 mg/ml and saline were used as positive and negative controls, respectively, in all subjects.

None of the control donors had a clinical history of allergic disease, and none had specific serum IgE or a positive SPT reaction to any of the common allergens of the Mediterranean area.

Allergens

Pollen from Pj (Allergon AB, Vålinge, Sweden) was extracted as previously described (15) and stored lyophilized at -20 °C until used. Working solutions (1 mg lyophilized material/ml RPMI 1640 medium (BioWhittaker, Walkersville, Maryland) were stored at -20 °C until used. The extracts contained all important allergens as evaluated by sodium dodecylsulfate-polyacrylamide gel electrophoresis immunoblotting. Chromatographic purification of profilins was done as previously described (16). Proteins co-eluted with profilin were removed by high-resolution chromatography in the SMART™ system (Pharmacia), as also described (16).

PBMC stimulation assay

PBMCs were isolated from freshly drawn heparinized blood by gradient centrifugation on Lymphoprep (Nycomed Pharma, Oslo, Norway), washed twice with PBS (bioMérieux, Marcy l'Etoile, France), and resuspended in X-VIVO 10 medium (BioWhittaker, Walkersville, Maryland) with L-glutamine, without antibiotics (referred to as medium). The PBMCs were stimulated with 10 μ g/ml of Pj profilin, in 96-well, round-bottomed plates with 1 $\times 10^5$ cells/well in 200 μ l medium/well. Optimal conditions for the proliferation assays were determined by pilot studies with allergen concentrations from 100 to 0.01 μ g/ml and harvest of the cells at day 3, 6 or 10 with PBMCs from patients and non-atopic donors. PBMCs in cultures without allergen were used as negative controls; PBMCs stimulated with 5 μ g/ml tetanus toxoid (TT) (bioMérieux, Lyon, France) or with 1/100 PHA (Wellcome, Beckenham, U.K.) were used as positive controls. Cells were cultured for 6 days in a humidified atmosphere at 37 °C, 5 % CO₂, followed by an 18-hour pulse in the presence of 0.5 μ Ci of ³H-thymidine (Amersham, U.K.) per well. The cells were harvested on to nitrocellulose filters and thymidine incorporation was determined by liquid scintillation counting (Betaplane, BTK, Wallac).

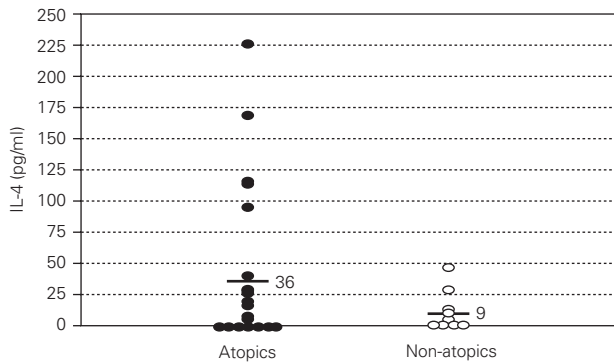


Figure 4.—IL-4 production by PBMCs of atopic patients (closed circles) versus non-atopic donors (open circles). Polyclonal cytokine production: PBMCs were incubated with PHA (1/100) for two days and the concentration of IL-4 was determined by ELISA. Atopic patients produced higher levels of IL-4 (mean 36 ± 59 SD) than non-atopic controls (mean 9 ± 15 SD) ($p = 0.001$).

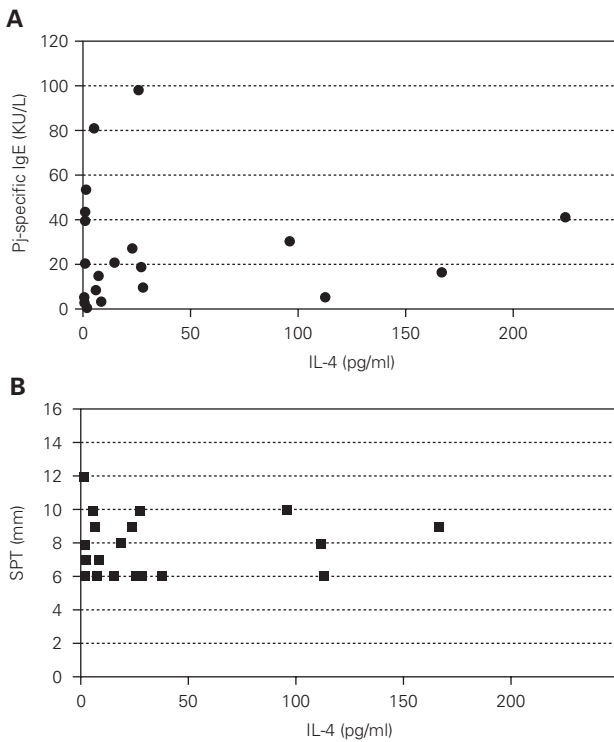


Figure 5.—Correlation between polyclonal IL-4 production and specific IgE (A) or SPT (B). Pj-specific IgE concentrations were determined in a CAP-SYSTEM. (Pharmacia, Upsala, Sweden) and expressed as KU/L. The concentration of IL-4 was determined by ELISA, and expressed as pg/ml on the supernatants of the cultures of PBMCs from Pj atopic patients stimulated with PHA (1/100) for two days. The Pj profilin wheal diameter are expressed in mm. Spearman rank order Correlation: A: $r = 0.003$. B: $r = 0.161$.

T-cell lines did not show cross-reactivity with another highly purified profilin (from Phl p) (fig. 6).

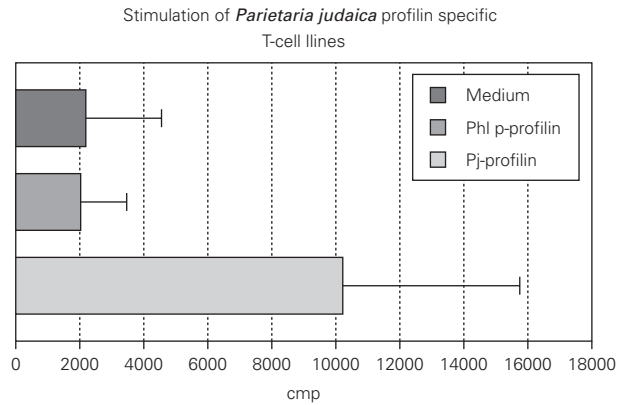


Figure 6.—Reactivity of Pj profilin specific T-cell lines with a highly homologous profilin from Phl p. Seven T-cell lines were established from the PBMCs of four Pj profilin atopic patients. Results are expressed as the mean of cpm of triplicate cultures, stimulated with Phl p profilin (mean 2045 ± 1433 SD), Pj profilin (mean 10269 ± 5477 SD), or medium alone (mean 2207 ± 2395 SD).

DISCUSSION

The main findings of our study are: (a) The patients allergic to Pj profilin had a significantly increased T-cell proliferative response to this profilin compared with non-atopic subjects. (b) The enhanced T-cell proliferation of the atopic patients was not correlated to skin reactivity or to specific serum IgE levels. (c) Specific Pj profilin T-cell lines did not show cross-reactivity with another highly purified profilin (Phl p profilin). (d) The group of atopic patients has a significantly increased polyclonal (PHA) production of IL-4, compared with non-atopic subjects, and (e) this increased production of IL-4 did not correlate with skin reactivity or specific serum IgE levels.

The enhanced T-cell responses to profilins in the atopic patients, although small, indicate an increased frequency of allergen-specific T-cells (17). The fact that enhanced profilin specific T-cell responses are found in the peripheral blood of patients suggests that the responding T-cells are inducing the production of the allergen-specific IgE by B cells and the subsequent skin reactivity. Therefore, a direct correlation between the level of T-cell reactivity and allergen-specific serum IgE and SPT could be expected. However, in this study neither specific serum IgE levels nor SPT diameters could be correlated to the level of profilin specific T-cell responses of the patients. In similar studies, we (18) and others (14), working with Phl p profilin and grass allergen extracts and immunoaffinity-purified group 5 allergens of the Poaceae family (Phl p, *Poa pratensis*, *Lolium perenne*) reached similar conclusions. In these works, neither it was found any

Stimulation index (SI) values were calculated as the ratio of cpm at the optimal antigen concentration in the stimulated cultures relative to the cpm in the unstimulated cultures. All results represent the mean values of triplicate cultures.

Cytokine production

PBMCs were thawed and stimulated in medium with 25 µg/ml of Pj profilin or 1/100 PHA (Wellcome, Beckenham, U.K.), in 96-well, flat-bottomed plates; 3×10^5 cells/well in 300 µl medium/well were used. The cells were cultured in humidified atmosphere at 37 °C, 5 % CO₂; PHA and Pj profilin supernatants were collected at days 2 and 7 respectively. IL-4 and IFN-γ concentrations in supernatants were determined by ELISA (Bender, Vienna, Austria) according to the manufacturer's description.

Establishment of Pj specific T-cell lines

T-cell lines specific to Pj profilin were established from PBMCs of 27 patients allergic to this profilin. Isolated PBMCs (1×10^6 /ml) were stimulated in 8 ml bulk cultures in 24-well flat-bottomed culture plates (Nunc, Roskilde, Denmark) with 10 µg/ml of profilin for 7 days. Subsequently, recombinant IL-2 (rIL2) (10 U/ml; Roche, Nutley, New Jersey, USA) was added and kept in culture for an additional 7 days.

T-cell stimulation assay

On day 14 after stimulation, the proliferative capacity of the T-cell lines was assessed: 2×10^4 T-cell blasts were incubated in triplicate in 200 µl cultures in the presence of 10^5 irradiated (2,500 rads) autologous PBMCs plus the appropriate profilin (10 µg/ml) in 96-well round bottomed microtiter plates for 48 hr at 37 °C, in a humidified atmosphere of 5 % CO₂, followed by an 18-hour pulse with 0.5 µCi of ³H-thymidine per well (Amersham), radionuclide uptake was measured by scintillation counting. Cultures with positive SI were selected.

Statistical analysis

Comparison of T-cell proliferation (expressed as SI) and cytokine production (expressed as pg/ml) between groups was done by Student's *t* test or

Mann Whitney U test to compare groups of data with non-normal distribution. Spearman rank order correlation was used to detect correlation between different data sets. All stochastic probabilities less than or equal to 0.05 were considered significant. Analyses were performed with SPSSWIN 6.1.3 (SPSS Inc., 1989-1995).

RESULTS

SPT and allergen-specific IgE

All patients had a positive SPT to both Pj crude extract and Pj highly purified profilin, and all but one had Pj specific IgE. No correlation was found between the patients' SPT wheal diameter (mean 7,75 ± 2,2 SD) and the concentration of specific IgE in serum (fig. 1). Total serum IgE levels ranged from 11 to 1,511 IU/ml (mean 228 ± 316 SD). Pj specific IgE levels range from 0.3 to 97 KU/L (mean 28.9 ± 27.2 SD).

Proliferative T-cell responses to allergens

Both patients and control subjects exhibited proliferative T-cell responses to the allergen extract with maximal stimulation ranged 1 to 100 µg/ml. To ensure that the results were not influenced by the variation in background counts, they were expressed as SI. The responses expressed as mean SI to crude extract and highly purified profilin were higher in the group of atopic patients than in the non-atopic subjects (fig. 2). Proliferative responses of patient's PBMC to Pj profilin were generally low, with SI values ranging from 1 to 6 (mean 2,04 ± 0.7 SD). This finding was in contrast with their SPT response to Pj profilin and high Pj specific IgE. Proliferative responses to TT and PHA were higher in the group of non-atopics than in patients, but the difference was not significantly (fig. 2).

To further analyze the Pj profilin-specific T cell reactivity 185 T cell lines were generated from the peripheral blood of 27 Pj profilin allergic patients. A substantial increase in the SI was achieved, but the majority of T cell lines still exhibited low reactivity against Pj profilin (median 4 ± 6.2 SD).

The level of profilin-specific T-cell proliferation did not correlate with specific Pj IgE concentrations (fig. 3A) or Pj profilin SPT (fig. 3B) of the individual patients.

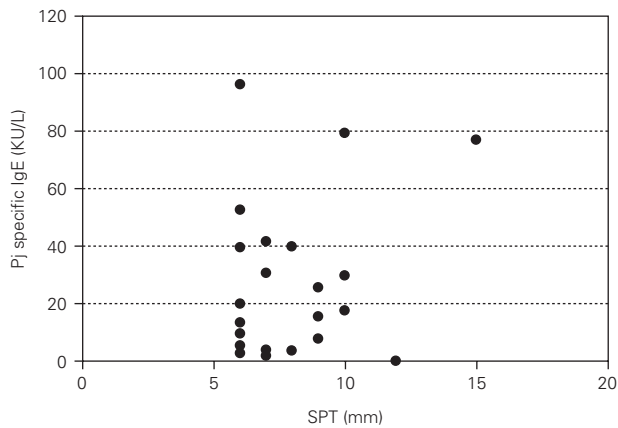


Figure 1.—SPT versus specific IgE. SPT results are expressed as Pj profilin wheal diameter in mm. Pj-specific IgE concentrations were determined in a CAP-SYSTEM. (Pharmacia, Uppsala, Sweden) and expressed as KU/L. Spearman rank order correlation: $r = 0.04$, $p = 0.82$.

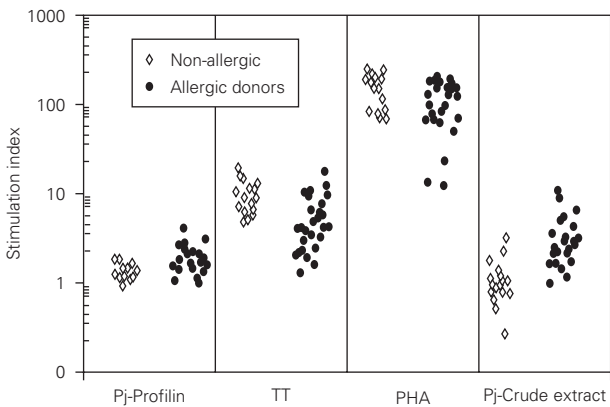


Figure 2.—Specific T-cell responses to profilin and crude extract from Pj. Results are expressed as SI obtained by stimulation of PBMCs from atopic donors (closed figures) or non-atopic controls (open figures). Mean values are significantly higher for Pj profilin in atopics (mean 2.04 ± 0.7 SD) than in controls (mean 1.47 ± 0.28 SD) ($p = 0.007$). Also, mean values are significantly higher for Pj crude extract in atopics (mean 3.47 ± 2.5 SD) than in controls (mean 1.17 ± 0.16 SD) ($p = 0.003$). Mean values are non significantly higher for TT in controls (mean 10.5 ± 4.2 SD) than in atopics (mean 5.7 ± 3.9 SD) ($p = 0.481$). Also, mean values are non significantly higher for PHA in controls (mean 152 ± 60.7 SD) than in atopics (mean 121 ± 59 SD) ($p = 0.98$).

Cytokine profiles of the allergen-specific T-cell response

To determine whether the cytokine production of the patients was consistent with the atopic phenotype, supernatants from profilin and PHA-stimulated PBMCs from patients and control subjects were assayed for production of IL-4 and

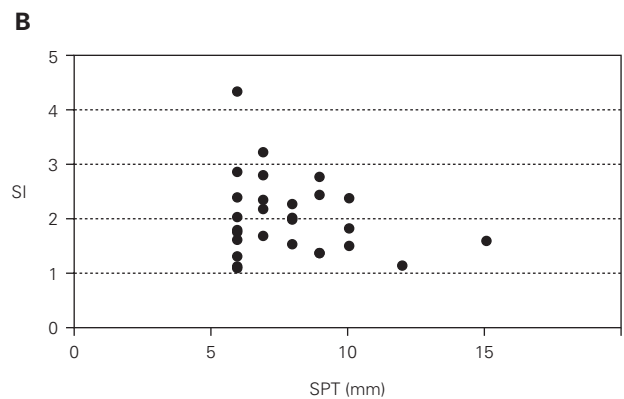
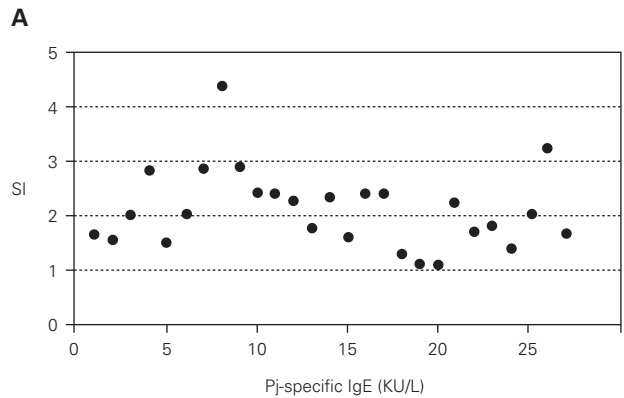


Figure 3.—(A). Specific T-cell responses to Pj profilin versus specific IgE. Results are expressed as SI obtained by Pj profilin stimulation of PBMCs from atopic donors and Pj-specific IgE (KU/L). The IgE concentrations were determined in a CAP-SYSTEM (Pharmacia, Uppsala, Sweden). Spearman rank order correlation: $r = 0.16$, $p = 0.432$. (B). Specific T-cell responses to Pj profilin versus SPT. Results are expressed as SI obtained by Pj profilin stimulation of PBMCs from atopic donors and Pj profilin wheal diameter in mm. Spearman rank order correlation: $r = 0.09$, $p = 0.620$.

IFN- γ . The production of IL-4 on PHA-stimulated PBMCs from atopic patients was higher than from control subjects ($p = 0.001$) (fig. 4), whereas the production of IFN- γ on the same conditions was not different ($p = 0.38$, results not shown). The production of IL-4 and IFN- γ by profilin-stimulated PBMCs was undetectable in the majority of patients and controls (results not shown). IL-4 polyclonal production level did not correlate with the specific Pj IgE concentration (fig. 5A) or Pj profilin SPT (fig. 5B) in the atopic patients.

Cross-reactivity of Pj and *Phleum pratense* profilins

To study the possible T-cell cross-reactivity we selected 7 of the better Pj profilin T-cell lines established from the PBMCs of four patients. This

correlation between the magnitude of the patients' T-cell responses and the skin prick test areas or specific serum IgE levels.

The small increase in the T cell response of the patients group, was in contrast to the positive results of the SPT, that were in general vigorous. Low responsiveness in the proliferation test were characteristics of Pj profilin when assayed in PBMCs and also with T-cell lines. A general defect in the proliferative capacity of lymphocytes in these patients could be excluded because the TT and PHA controls induce good proliferative responses. Similar low T cell responsiveness has been described with Bos d 2 (19), s1-casein (20) and Fel d1 (21). The explanation of how a low T-cell responsiveness can sustain and elevated SPT in the group of Pj profilin atopic patients, remains especulative at this moment, but could be related to the fact that profilins are ubiquitous and highly conserved proteins: there is a 70 % of aminoacid sequence homology between human and plant profilins.

We were unable to detect the production of IL-4 (a typical T_{H2} cytokine) and IFN- γ (a typical T_{H1} cytokine) when PBMCs from atopic patients and non-atopic controls were stimulated by Pj profilin because the levels of the cytokines in the supernatants of the cultures were too low for the sensitivity of the method. However, the increased production of IL-4 and the tendency to a reduced production of IFN- γ , due to a polyclonal stimulation (PHA) of peripheral T-cells of the atopic patients, reflects their allergic phenotype. Several researchers have reported cytokine production patterns in PBMCs activated by non-specific stimulation in atopic diseases. It has been shown that in vitro IFN- γ production by PHA-stimulated PBMCs is greatly diminished in atopic dermatitis patients compared with non-atopic healthy controls (22). In addition, it has also been demonstrated that the activation of PBMCs from atopic patients with high serum IgE induce higher levels of IL-4 synthesis and lower levels of IFN- γ than the levels produced by PBMC from healthy controls, suggesting that enhanced IL-4 production and reduced IFN- γ production are associated with the elevated serum IgE levels in atopic patients (23, 24).

Recently, several plant profilins have been cloned and sequenced (25-29). Their deduced amino acid sequences show a high degree of homology. However, the T-cell lines raised against Pj profilin did not show any reactivity with Phl p profilin. Even though these data were obtained from experiments with a limited number of T-cell lines, the results indicate the importance of using T-cell lines (or clones) and highly purified (or recombinant) allergens

in order to stablish the cross-reactivity between allergens at the T-cell level.

In conclusion, the results of the present study show that Pj profilin specific proliferative T-cell response and the polyclonal production of IL-4 are higher in patients than in the group of control subjects. This suggests a role for Pj profilin in the induction of T-cell-driven immunological reactions. The lack of correlation between the skin reactivity and IgE-specific concentration, T-cell reactivity or IL-4 production most likely reflects the complexity of the skin reactivity, whereas the fact that the proliferative T-cell response did not correlate to the patients serum IgE suggests that at least some of the responding T-cells may be involved in immune reactions other than the support of IgE production. That profilins are ubiquitous and highly conserved proteins could be the explanation of the low T-cell responsiveness and elevated SPT, in our group of patients. Pj profilin specific T-cell lines did not show cross-reactivity with a highly homologous profilin from Phl p (14).

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RESUMEN

Antecedentes: La enfermedad alérgica originada por *Parietaria judaica* (Pj) ha sido ampliamente documentada en la zona Mediterránea. Las profilinas han sido identificadas como proteínas alergénicas de amplia distribución. El papel de la profilina de Pj en la respuesta inmunológica específica en pacientes sensibilizados a la Pj es desconocida.

Métodos: Se determinaron en todos los pacientes (n = 28) y controles normales no atópicos (n = 18) el test de reactividad cutánea y los niveles tanto de IgE específicas como totales en suero. Se aislaron células mononucleares de sangre periférica (PBMC) en ambos grupos y fueron estimuladas con el extracto crudo o con la profilina altamente purificada de Pj. Se determinaron también la producción de citocinas de tipo I y de tipo II, con estimulaciones específicas o policlonales en pacientes y controles. Establecimos líneas de células T específicas por la profilina de Pj y evaluamos la reactividad cruzada con

otra profilina altamente purificada de *Phleum pratense* (Phl p).

Resultados: Los pacientes sensibilizados a la profilina de Pj muestran un ligero pero significativo incremento en la respuesta proliferativa T frente a la profilina respecto con el grupo de controles no alérgicos. La producción de IL-4 y IFN- γ en respuesta a estímulos específicos fue indetectable. Sin embargo, la producción de IL-4 en respuesta a un estímulo policlonal (PHA), fue significativamente mayor en pacientes atópicos que en los controles no alérgicos. La respuesta celular T no se corresponde ni con la magnitud de la respuesta en la reactividad cutánea a la profilina de Pj ni con los niveles de IgE en suero específicos para la Pj. La producción de IL-4 frente a un estímulo policlonal (PHA), no se correlaciona con la reactividad cutánea a la profilina ni con los niveles de IgE en suero. Las líneas específicas T testadas no mostraron ninguna reactividad cruzada con la profilina de Phl p.

Conclusiones: Nuestros resultados sugieren que la profilina de Pj es responsable en parte de la respuesta mediada por células T en pacientes

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alérgicos a la Pj. La elevada reactividad cutánea de estos pacientes, se acompaña de un pequeño incremento en la respuesta de células T a esta profilina. La respuesta es muy específica porque las líneas de células T no presentaron respuesta cruzada con la profilina altamente homóloga de Phl p. La falta de correlación entre la respuesta proliferativa de las células T y la producción policlonal de IL-4 con los niveles de IgE específicos en suero y la reactividad cutánea probablemente indica que algunas de las células T respondedoras estarían involucradas en una reacción inmunitaria diferente a la que promueve la producción de IgE.

Palabras clave: Célula T. Profilinas. Prueba de reactividad cutánea. IgE específica. IL-4. IFN- γ . *Parietaria judaica*.

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