

Diversity of asparagus allergy: clinical and immunological features

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Summary

Background Asparagus (*Asparagus officinalis*) is an extensively grown and consumed vegetable. To a lesser extent than other Liliaceae vegetables, allergic contact dermatitis (ACD) due to asparagus has been reported. However, only a few case reports of asparagus IgE-mediated allergy have been published. In a previous study, we demonstrated that two lipid transfer proteins (LTPs) (Aspa o 1.01 and Aspa o 1.02) were relevant allergens of asparagus.

Objective We retrospectively analysed the 27 patients diagnosed with asparagus allergy during the last 5 years. All of them reported adverse symptoms after either asparagus ingestion or handling. We describe their clinical features and evaluate whether they were associated to immunological findings (immunoblot pattern and skin reactivity to LTPs).

Methods Patients underwent skin prick and patch tests with standard panels of vegetables and aeroallergens. Besides crude asparagus extract, two purified LTPs were prick and patch tested. Total and specific IgE measurements and asparagus extract IgE immunoblotting were performed. Patients reporting asthma symptoms underwent specific inhalation challenge to asparagus.

Results Of the 27 subjects, eight had ACD, 17 had IgE-mediated allergy and two had both ACD- and IgE-mediated allergy. Positive patch tests with the crude asparagus extract but not with LTPs were observed in subjects with ACD ($n = 10$). Of 19 patients with IgE-mediated disease, 10 had contact urticaria after asparagus handling. Of them, five subjects and five others without skin allergy showed respiratory symptoms; of them, eight were diagnosed with occupational asthma confirmed by positive asparagus inhalation challenge, whereas the remaining two had isolated rhinitis. Four patients suffered from immediate allergic reactions related to asparagus ingestion (food allergy); three of them reported anaphylaxis whereas the other had oral allergic syndrome. Positive IgE immunoblotting (bands of 15 and 45–70 kDa) was observed in 10 subjects. Of 10 subjects with positive prick test to LTPs, six showed bands at 15 kDa. Either IgE-binding bands or positive prick tests to LTPs were observed in asthma (62%) and anaphylaxis (67%).

Conclusion Asparagus is a relevant source of occupational allergy inducing ACD and also IgE-mediated reactions. Severe disease (anaphylaxis or asthma) is common and LTPs seem to play a major role. The clinical relevance of LTP sensitization among patients with mild disease or symptom-free subjects should be addressed in prospective studies.

Keywords asparagus allergy, food allergy, immunoblotting, lipid transfer proteins, occupational asthma, specific inhalation challenge

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Introduction

Asparagus (*Asparagus officinalis*), garlic, onion and leek are members of the Liliaceae family. Allergic contact dermatitis (ACD) due to these vegetables is quite common among housewives [1] and catering workers [2, 3]. In some countries, garlic is considered to be the next allergen inducing ACD among housewives after nickel [1]. To a lesser extent,

asparagus may give rise to occupational ACD among housewives, cooks and cannery operators [4]. Liliaceae vegetables also induce immediate IgE-mediated reactions and again, garlic is the most commonly implicated [5–7]. With regard to asparagus, only a few case reports of IgE-mediated contact urticaria [8] and occupational rhinitis and asthma [9, 10] have been reported. IgE-mediated food allergy due to cooked asparagus ingestion has also been described [11].

In 2000, more than 4 million tonnes of asparagus were grown in the world. China was the first producing country with 3.6 million tonnes and Spain was the fourth one with 61 000 tonnes (www.cci.org.co). In our region, this sector

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produces about 1800 tonnes/year and employs 2315 harvesters (from April to mid-July) and 1147 canning processing workers (www.denominacionesnavarra.com/esparrago). During the last 5 years, we diagnosed 27 subjects with asparagus allergy in the form of either ACD or IgE-mediated reactions. We demonstrated that lipid transfer proteins (LTPs), profilin and high-molecular-weight (MW) glycoproteins harbouring cross-reacting carbohydrate determinants were relevant allergens among these patients [12]. In the present study, we describe the clinical pictures secondary to asparagus allergy and their relationship with both immunoblotting pattern and skin sensitization to asparagus LTPs (Aspa o 1.01 and Aspa o 1.02) [12].

Patients and methods

Study protocol

The 27 patients diagnosed of asparagus allergy during the last 5 years, all of them reporting adverse symptoms following either its ingestion or handling, underwent skin prick tests (SPTs), patch tests and serum IgE measurements. Asparagus extract immunoblotting was performed with serum samples from patients with detectable asparagus-specific IgE. Subjects reporting bronchial symptoms related to asparagus underwent specific inhalation challenge (A-SIC). The hospital ethics committee approved the study protocol and informed consent was obtained from all participants.

Allergen extracts and purified asparagus LTPs

The extracts of vegetables (endive, artichoke, onion, cabbage, tomato, lettuce, asparagus, spinach, carrot, celery, potato, parsley, leek, garlic and pepper) were prepared at our laboratory. Each vegetable was liquefied and then lyophilized. The lyophilized material was reconstituted until original volume with 50% glycerinated phosphate buffered saline (PBS) for SPT. Asparagus extract for A-SIC was obtained by extracting freeze-dried asparagus (10% w/v) with PBS buffer at 4°C for 1 h. After centrifugation, the supernatant was extensively dialysed against PBS and filtered (0.22 µm). Freeze-dried asparagus, onion and garlic extracts were mixed at 10% in petrolatum for patch tests.

Purified asparagus allergens Aspa o 1.01 and Aspa o 1.02 (LTPs) were obtained as described [12]. Briefly, freeze-dried asparagus was ground and extracted with PBS. It was then fractionated by cation-exchange chromatography on an A cell Plus CM Waters SepPak cartridge (Waters Corp, Milford, MA, USA), and the retained LTP-enriched fraction was further separated by reverse-phase high-performance liquid chromatography [12]. Two peaks containing LTPs were identified and characterized by N-terminal amino acid sequencing [12]. Additionally, an asparagus protein preparation, hereafter designated as a non-retained (NR) fraction was also obtained by subjecting the asparagus PBS extract to cation-exchange chromatography [12]. The NR fraction virtually lacked LTPs, as judged by the absence of band recognized by rabbit polyclonal antibodies anti-Pru p 3 [12]. Both LTPs were diluted in PBS at 20 µg/mL for SPT and patch tests. The NR fraction was also patched at 2 mg/mL in PBS.

Skin tests

SPTs were performed as previously described [13] with a panel that included the most common inhalant allergens in our environment (mites, pollens, moulds, cat and dog dander and latex) (ALK-Abelló, Madrid, Spain), our panel of vegetables and the two asparagus purified LTPs (20 µg/mL) [12]. The resulting weals were transferred to a millimetre-squared paper, where areas were measured and expressed in mm². It was considered that one patient was sensitized to LTPs when either Aspa o 1.01 or Aspa o 1.02 was positive (area ≥ 7 mm²). Atopy was defined as positive SPT to one or more of the common aeroallergens.

Patch tests were carried out according to international guidelines [14] with a standard battery (True Test[®], ALK-Abelló), our extracts of asparagus, garlic and onion and with the commercial contact allergen of garlic: diallyldisulphide (Marti Tor[®], Barcelona, Spain). The two characterized LTPs (20 µg/mL) and the NR fraction of the asparagus (2 mg/mL) extract were also patched. The results were evaluated at 48 and 96 h. Ten healthy controls gave negative results to both prick and patch tests performed with our own extracts.

IgE measurement

The total serum IgE and asparagus-specific IgE were measured by the Pharmacia CAP system IgE fluoroenzyme immunosorbent assay (Pharmacia diagnostics, Uppsala, Sweden).

Immunoblotting

Immunodetection was carried out by means of AlaSTAT AlaBLOT-specific IgE (DPC, LA, USA). After electrophoresis separation in 4–10% gradient polyacrylamide gel, the resolved asparagus extract was electrotransferred onto nitrocellulose membranes, which were cut into strips provided in the AlaBLOT kit. Nitrocellulose strips were incubated with the individual samples of patients' serum at 1:3 dilution in PBS, at room temperature, for 2 h. After washes, strips were incubated with alkaline phosphatase-labelled anti-human-IgE monoclonal antibody for 30 min. After new washes, the substrate solution was added and allowed to react until an appropriate intensity of bands was obtained. MW of the stained bands was calculated by the lot-specific formula provided with the strips. Immunoblotting was negative in six patients with undetectable levels of asparagus-specific IgE.

Asparagus-specific inhalation challenge (A-SIC)

Every lung function test was performed with the same spirometer (CPR, Medical Graphics Corp., St Paul, MN, USA). Baseline forced expiratory volume in 1 s (FEV₁) values ≥ 70% predicted normal were required for inhalation tests. Challenges were carried out while patients were clinically stable and not taking anti-asthmatic drugs, with the exception of inhaled short-acting β₂-agonist drugs used 'as needed', which were withdrawn 8 h before the test. The peak expiratory flow rate (PEFR) was recorded (Mini-Wright peak flow meter, Clement Clark International Ltd, London, UK) before starting the A-SIC. Skin tests with 2-fold dilutions of the allergen extract were performed to enable

the selection of a safe initial dose (concentration producing a 3-mm diameter weal). Allergen dilutions at 2-fold increasing concentrations were administered by a dosimeter (MEFAR s.r.l.; MEFAR; Borezzo, Italy) programmed to deliver five inhalations of 1 s each. The dosimeter was always attached to the same nebulizer calibrated to deliver 10 µL of solution in each second. Patients were carefully instructed to inhale the solution slowly from the residual volume to the total lung capacity, and 10 µL of solution was administered in each breath. Before starting the challenges, patients inhaled a diluent. A variability in FEV₁ values <5% between baseline and post-diluent FEV₁ values was required. FEV₁ values were recorded 10 min following each allergen concentration. The test finished either when a fall in FEV₁ values ≥20% from the post-diluent value was achieved, or when the highest concentration of allergen (10% w/v) was inhaled. Early asthmatic responses were expressed in terms of the allergen-PC₂₀ (provocative concentration of allergen needed to decrease FEV₁ by 20% of the baseline values). PEFR rates were recorded hourly during the following 10 h. The late asthmatic response was expressed as the percentage of maximal PEFR decrease from the baseline values and considered to be positive when ≥25% [15]. A-SIC was negative in four control asthma patients who were not sensitized to asparagus (data not shown).

Statistical analysis

The SPSS for windows 8.0 program (SPSS, Chicago, IL, USA) was used for statistical analysis. Contingency tables were elaborated, and the χ^2 test was used to compare the characteristics among groups. A *P*-value lower than 0.05 was considered to be significant.

Results

The demographic and clinical features of the patients are shown in Table 1. Several clinical pictures related to asparagus handling were observed: ACD (37%), contact urticaria (37%), rhinitis (37%) and occupational asthma (30%). Asparagus ingestion induced immediate allergic reactions in 15% of the patients.

Allergic contact dermatitis (ACD)

Positive patch tests with asparagus but negative with garlic, diallyl disulphide and onion were observed in the 10 subjects diagnosed with ACD. All of them exhibited positive patch tests with the NR asparagus extract fraction, but with neither Aspa o 1.01 nor Aspa o 1.02. They were occupationally

Table 1. Demographic features and diagnosis of the subjects included

Subject	Age/sex	Atopy	Job	TlgE (kU/L)	A-IgE (kU/L)	A-SPT	LTP-SPT	A-PT	LTP-PT	NRF-PT	IB (<15/>15)	Diagnosis
1	23/M	+	CP	1240	12.7	+	+	—	—	—	+/+	RH/OA/CU
2	31/F	+	HW	635	9.17	+	—	—	—	—	—/—	RH/OA
3	35/M	+	Cook	2441	1.98	+	—	—	—	—	+/+	RH/OA/CU
4	55/F	+	CP	393	0.45	+	—	—	—	—	—/—	RH/OA/CU
5	45/F	—	HW	28	1.58	+	—	—	—	—	—/—	RH/OA/CU
6	42/F	+	HW	249	4.77	+	+	—	—	—	+/+	RH/OA
7	26/F	+	GR	231	9.7	+	+	—	—	—	+/+	RH/OA
8	34/F	—	CP	70	4.47	+	+	—	—	—	+/—	RH/OA/FA ^{AN}
25	44/F	—	HW	88	0.93	+	ND	—	—	—	ND	UC/RH
9	28/M	+	GR	1773	12.0	+	+	—	—	—	+/+	FA ^{AN}
10	18/M	+	NR	392	0.43	+	—	—	—	—	—/+	FA ^{AN}
11	32/M	+	NR	1857	1.17	+	+	—	—	—	—/—	FA ^{OAS}
12	21/F	+	CP	155	1.18	+	+	—	—	—	—/+	CU
13	9/M	+	NR	320	9.25	+	+	—	—	—	+/—	CU
14	19/F	+	CP	366	0.41	+	—	—	—	—	—/—	CU
15	36/F	—	HW	66	1.24	+	+	—	—	—	—/—	CU
16	68/F	—	HW	47	0.68	+	—	—	—	—	—/—	CU
26	48/F	—	CP	11	<0.35	+	ND	+	—	+	ND	RH/ACD
17	33/F	—	CP	40	1.57	+	+	+	—	+	—/—	ACD
18	23/M	—	CP	355	0.52	+	—	+	—	+	—/+	ACD
19	50/F	—	CP	105	<0.35	—	—	+	—	+	—/—	ACD
20	50/F	—	CP	6	<0.35	—	—	+	—	+	—/—	ACD
21	19/F	—	CP	16	<0.35	—	—	+	—	+	—/—	ACD
22	40/F	—	HW	23	<0.35	—	—	+	—	+	—/—	ACD
23	49/F	—	CP	76	<0.35	—	—	+	—	+	—/—	ACD
24	35/F	—	CP	14	<0.35	—	—	+	—	+	—/—	ACD
27	78/F	—	HW	58	<0.35	—	—	+	—	+	ND	ACD

TlgE, total serum IgE; A, asparagus; SPT, skin prick test; PT, patch tests; NRF, non-retained fraction of the asparagus extract; LTPs, lipid transfer proteins; IB, immunoblotting (bands at 15 kDa/bands higher than 15 kDa); CP, canning processor; HW, housewife; GR, grocery; NR, non-related; CU, contact urticaria; RH, rhinitis; OA, occupational asthma; FA, food allergy (FA^{AN}, anaphylaxis, FA^{OAS}, oral allergic syndrome); ACD, allergic contact dermatitis; M, male; F, female; ND, not done. Subjects' numeration corresponds to that employed in both, Table 2 and Fig. 1. Patients # 1–18 were included in our previous report [12].

exposed to asparagus (80% canning operators and 20% housewives), tolerated its ingestion and were not atopic. Seven subjects reacted to standard patch tests, three to rubber additives (carbamates and thiuram) and the remaining four to metals (nickel and cobalt).

IgE-mediated allergy

Nineteen patients showed a positive SPT and specific IgE to asparagus (Table 1). Two of them (nos. 17 and 18) had a positive asparagus patch and no other symptoms other than ACD. One additional patient (no. 26), diagnosed with ACD and rhinitis, had a positive asparagus patch and prick tests but undetectable levels of specific IgE. Immunoblotting showed bands of approximately 15 kDa (70%) and 45–70 kDa (80%) (Fig. 1). Low-MW bands were observed in seven patients; six of them also exhibited positive SPT with LTPs. Alternatively, four patients in whom immunoblotting was negative showed positive SPT for LTPs (Table 1).

Ten patients were diagnosed of contact urticaria (erythema, pruritus and weals exclusively at the contact skin areas). Atopy and sensitization to other vegetables was observed in 60% and 40% (nos. 1, 3, 13 and 15). Five patients had isolated cutaneous symptoms, whereas the remaining also had respiratory symptoms (Table 1). All the 10 patients presenting respiratory symptoms underwent A-SIC that was well tolerated in all instances. This test was positive among the eight subjects who, besides rhinitis, reported bronchial symptoms related to asparagus handling. Isolated immediate response was observed in five patients, whereas the remaining three subjects presented immediate and late asthma responses (dual responders) (Table 2). A-SIC was negative in the two patients who reported isolated rhinitis symptoms. Seven patients were atopic and five subjects (nos. 1, 3, 6, 7 and 8) were sensitized to other vegetables. The immunoblotting was positive for five patients with occupational asthma; all of them exhibited low-MW bands (Fig. 1) and SPT with LTPs were positive for four of them (Table 1).

With the exception of subject no. 8, a non-atopic woman who presented with one anaphylaxis episode (dyspnoea,

angiooedema and generalized urticaria) requiring hospitalization, the remaining subjects with rhinitis, asthma or contact urticaria tolerated the ingestion of boiled asparagus. Three additional atopic subjects experienced food allergy in the form of anaphylaxis (nos. 9 and 10) or oral allergy syndrome (no. 11). Immunoblotting was positive in the three patients with anaphylaxis: two of them exhibited low-MW bands and positive SPT with LTPs. High-MW bands were observed in the immunoblotting from subject nos. 9 and 10. Subject nos. 8 and 9 were also sensitized to onion and garlic. Patient no. 11 was also sensitized to nuts and fruits and, although the immunoblotting was negative, he had a positive SPT with LTPs.

Low-MW bands were most frequently observed among subjects exhibiting a positive SPT to LTPs ($P = 0.005$) and among patients with more severe disease (anaphylaxis or occupational asthma) ($P = 0.005$). In contrast, these later subjects did not show a higher degree of skin reactivity to LTPs.

Discussion

We present the 27 subjects diagnosed with asparagus allergy in our outpatient clinic during the last 5 years. The skin, in the form of either ACD or contact urticaria, and the airway were the most commonly affected organs (74% and 37%), whereas food allergy was present in 15%. As reported [4], ACD was a rather common manifestation; however, more than half of the patients had immediate IgE-mediated reactions related to their jobs, which contrasts with the few case reports available in the literature.

Most ACD patients were canning operators and showed eczema lesions located at the dorsal and lateral surface of fingers and flexural superficies of the arms, affecting mainly the left side. Such a distribution of lesions is highly suggestive of asparagus eczema. All the ACD patients showed positive patch tests with both the asparagus extract and the NR fraction of the asparagus extract. Patch tests with Aspa o 1.01, Aspa o 1.02, onion, garlic and diallyl-disulphide were

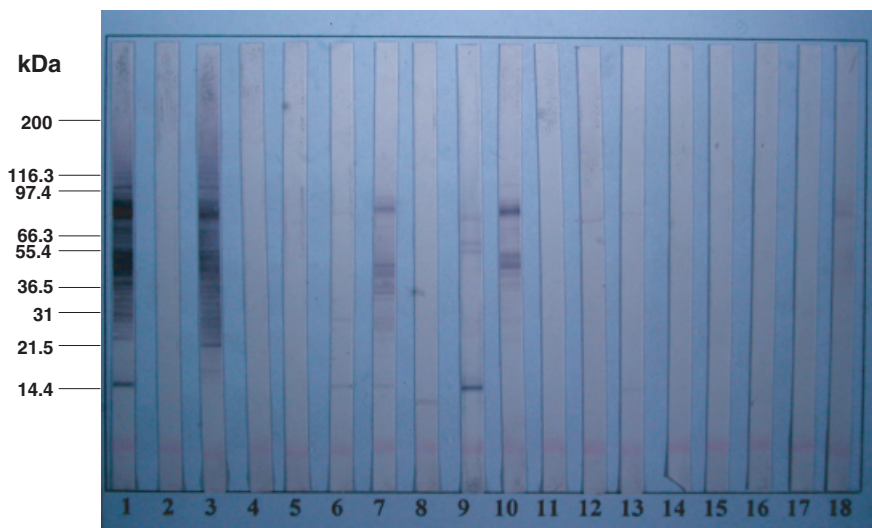


Fig. 1. Individual results of the asparagus IgE immunoblotting. Patients numbers (on the bottom of the picture) correspond to those exhibited in Tables 1 and 2.

Table 2. Results of asparagus-specific inhalation challenge among patients diagnosed with occupational asthma

	FEV ₁ (%)	A-PC ₂₀ (mg/mL)	LAR (%)
1	112	0.92	8
2	83	0.94	26
3	75	4.6	26
4	115	4.8	13
5	79	0.46	6
6	80	1.47	14
7	79	0.23	17
8	95	0.28	33

A-PC₂₀, asparagus-PC₂₀ values; LAR, late asthma response (percentage of drop in PEF values); FEV₁, forced expiratory volume in 1 s. Subjects' numeration corresponds to that employed in both, Table 2 and Fig. 1.

always negative. It could be argued that the negative patch with LTPs might be due to the concentration used (20 µg/mL). However, this concentration is the same naturally present in the entire asparagus extract [12] and thus, it is more likely that some other allergen different from LTPs is responsible for asparagus ACD, which agrees with reports showing the plant growth inhibitor, 1,2,3-tritiane-5 carboxylic acid, as such an allergen [4]. In contrast to some reports that have described systemic dermatitis related to garlic ingestion [16], all the ACD patients tolerated asparagus. The sensitization to rubber additives that presented three subjects would be likely due to the utilization of gloves to protect the hands from irritants and allergens at work. The wet features of the work and the use of stainless cooking instruments might account for sensitization to metals in the four patients from our study [17]. ACD patients were instructed to protect their hands and arms with long vinyl gloves, perfectly sealed at the upper extreme and could, in that way, continue safely with their jobs.

Of the three patients in the ACD group who showed either positive SPT or specific IgE to asparagus, only one (no. 26) had immediate symptoms (rhinitis). In contrast, specific IgE and SPT (but not patch tests) were always positive among patients with contact urticaria, rhinitis, asthma or food allergy. These subjects exhibited a higher frequency of atopy and sensitization to other vegetables than the ACD group, suggesting a clear separation between delayed contact allergy reactions (ACD) and IgE-mediated disease.

A few case reports of patients with respiratory symptoms due to asparagus have been documented [9, 10]. It is remarkable that in our study, eight out of the 10 patients who reported immediate rhinitis also had lower airway symptoms and were diagnosed of occupational asthma on the basis of positive asparagus inhalation challenge that elicited early and dual asthma responses in five and three patients, respectively. The substantial rate (33%) of housewives presenting either contact urticaria or respiratory allergy might be due to our regional culinary habits, since asparagus is usually peeled and boiled at homes. In keeping with the observations regarding garlic [18], subject no. 8, a woman previously diagnosed of occupational asthma had one anaphylaxis episode after eating canned asparagus. Three additional patients suffered from asparagus food allergy and it is notable that anaphylaxis was the most frequent clinical

presentation. We acknowledge that double-blind placebo-controlled food challenge is the 'gold standard' for the confirmation or refutation of any history of an adverse reaction to a food [19]. This challenge, however, can be omitted when one patient with food-specific IgE has a convincing history of a severe allergic reaction to the food [19]. Nevertheless, and in keeping with previous reports [9, 10], most subjects with contact urticaria or respiratory allergy tolerated the ingestion of boiled asparagus, which could be due to the inactivation of the incriminated allergens, by either heating or the digestive process. It is also possible that allergens are localized at the superficial layers of the vegetable that are removed before consumption.

LTPs constitute a family of polypeptides involved in plant defence mechanisms. They are widely distributed among the plant kingdom and have been identified as major allergens in many vegetables, including peach (Pru p 3) [20]. Thus, LTPs, together with profilins, are considered to be relevant panallergens and, whereas the former have been related to generalized and severe symptoms, profilins have been implicated in the less severe pollen-fruit syndrome [21, 22]. In our previous study [12], crude asparagus extract immunoblot showed bands at 15 kDa, which were the main IgE-binding proteins of the extract, and at 45–70 kDa. The high-MW bands likely corresponded to glycoproteins, but it was not possible to determine whether profilin or LTPs accounted for the low-MW ones. The anti-Pru p 3 reactivity observed when cation-exchange chromatography was applied to the extract [12] and the skin reactivity to purified LTPs observed in 86% of the patients with low-MW bands at immunoblot suggest that LTPs may be partially responsible for the allergenic activity of these bands.

Both immunodetection of low-MW bands and skin reactivity to LTPs were observed in two out of the three patients with anaphylaxis, four of the eight patients with occupational asthma, but only in one of the eight patients with mild symptoms (rhinitis, contact urticaria or oral allergy syndrome). According to these data, LTPs might play a role in the most severe symptoms: anaphylaxis [23] and occupational asthma. On the other hand, the lack of reactivity for LTPs observed in a number of these patients suggests that other asparagus proteins may also be implicated [10].

Alternatively, three patients with mild symptoms (nos. 11, 12 and 15) and one symptom-free subject (no. 17) exhibited skin reactivity to LTPs without evidence of low-MW bands at immunoblot. Such discordance could be attributed to the fact that LTP concentration in the entire conventional asparagus extract might be insufficient to elicit one band at immunoblot [24]. Whether skin sensitization to LTPs denotes a subgroup of subjects at risk for developing severe reactions should be addressed in prospective studies.

In our region, asparagus is a causative agent of occupational disease giving place to ACD (37%), and also to IgE-mediated reactions that occur in a considerable number of patients (52%), which are in contrast to the limited number of case reports published. Given the large asparagus world-wide production, our data suggest that IgE sensitization might be seriously underdiagnosed. The clinical approach to both diseases is different. Provided adequate protective measures, ACD patients can safely continue their labour activity. In contrast, discontinuation of allergenic exposure at the job

should be encouraged in patients with occupational IgE-mediated disease, giving rise to important socio-economic consequences.

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