

Characterization of allergens in plant-derived spices: Apiaceae spices, pepper (Piperaceae), and paprika (bell peppers, Solanaceae)

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Allergens in spices from the botanic families Apiaceae, Piperaceae, and Solanaceae were characterized. IgE-immunoblotting and IgE-inhibition experiments revealed broad cross-reactivity among spices and to pollen-derived proteins. Moreover, monoclonal antibodies raised against cross-reactive pollen allergens (profilin, Bet v 1) detected homologous proteins in almost every spice source analyzed. It can be concluded that allergy to spices rarely represents an autonomous sensitization, but is rather a consequence of pollen allergy on the basis of immunologic cross-reactivity.

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Immediate-type (type I) and delayed-type (type IV) allergy to spices is not rare (1–4). In particular, mugwort- and birch-pollen-sensitive individuals have been reported to suffer from adverse reactions to spices, obviously due to the presence of cross-reactive allergens in the respective plants (5–8). However, it has to be considered that spices also contain a number of pharmacologically active and/or toxic compounds, thereby possibly causing irritation and inflammation leading to intolerance reactions, which must not be mistaken as food allergy (9). Therefore, it is necessary to reveal and to characterize homologous proteins in plant-derived foods of different origins, which, due to immunologic cross-sensitization, induce allergic reactions in predisposed (atopic) individuals (10, 11). Spices are potentially dangerous food allergens because their presence in a dish is in many cases not obvious to the patient, and because minute amounts of hidden allergen can cause systemic reactions and even anaphylactic shock (12). In this paper, the presence of cross-reacting – and species-specific – allergens in spices belonging to the taxonomic families of Apiaceae, Piperaceae, and Solanaceae is discussed.

Material and methods

Extraction of spices, SDS-PAGE, and IgE immunoblotting were performed as previously described (6, 7). Recombinant pollen allergens (birch profilin: rBet v 2 and rBet v 1) for inhibition experiments were purchased from Biomay, Linz, Austria. Monoclonal antibodies against Bet v 1 and profilin used in immunoblots were detected by sheep-anti-mouse immunoglobulin (Amersham International Ltd, UK), as previously described (6).

Results and Discussion

Allergens in Apiaceae spices: anise, coriander, cumin, and fennel

Mugwort- and birch-pollen-allergic individuals frequently report adverse reactions after consumption of celery (*Apium graveolens*) or spices also belonging to the Apiaceae family. In this connection, the term “mugwort-celery-spice-syndrome” has been proposed by Wüthrich et al. (5). More recently, the cross-reactive allergens between mugwort, birch, and celery have been characterized (13, 14). As

expected, a high percentage of sera from spice-sensitive patients displayed IgE reactivity to the homologous allergens in anise, coriander, cumin, and fennel; i.e., allergens in the molecular weight range of 14–17 kDa and proteins of 50–60 kDa (Fig. 1) (6). The 14-kDa protein belongs to a group of ubiquitous cell proteins, the profilins (15). Profilin allergens are present in all members of the Apiaceae family, as well as in birch and mugwort pollen. Thus, preabsorption of specific IgE by recombinant birch profilin (rBet v 2) led to inhibition of IgE binding to the homologous allergens in spice extracts. Moreover, a monoclonal antibody created against celery profilin recognized a cross-reacting molecule in all extracts tested. The 17-kDa molecule represents a molecule with cross-reactivity to Bet v 1, the major birch-pollen allergen. BIP 1, a monoclonal antibody raised against Bet v 1, detected a cross-reacting antigen with corresponding molecular weight in all apiaceous spices tested. The high-molecular-weight IgE-binding pattern (Fig. 1) possibly represents a recently cloned ubiquitous plant enzyme, phosphoglyceromutase (16). Moreover, the presence of IgE reactivity to – still undetermined – carbohydrate determinants of food glycoproteins is under discussion (17, 18). Aqueous extracts from the spices were prepared from materials as they are used for consumption. In dried form, the IgE-binding capacity of anise and fennel was higher than the allergenicity of coriander and cumin (6); however, the allergen content in differently processed preparations or in fresh plants may vary.

Allergens in pepper (*Piperaceae*)

For the analysis of IgE-binding proteins in pepper extracts, a pooled extract from green and black peppercorns was used in immunoblot experiments. Testing sera from patients suffering from “mugwort-celery-spice-syndrome” (5) revealed two major allergens of 28 and 60 kDa, respectively. Fifteen out of 16 (93%) of the tested sera displayed IgE binding to these molecules. The molecules could not be identified as homologues of the birch-pollen major allergen Bet v 1 or of profilin. However, preincubation of sera with complete extracts of birch pollen, mugwort pollen, or celery tuber extract led to complete blocking of IgE binding, again indicating the existence of cross-reactive allergens in all these sources (7).

Allergens in bell peppers (*paprika*; *Solanaeae*)

Fresh bell peppers and dried bell-pepper fruits (paprika) are frequently the cause of adverse reactions to food in patients suffering from the

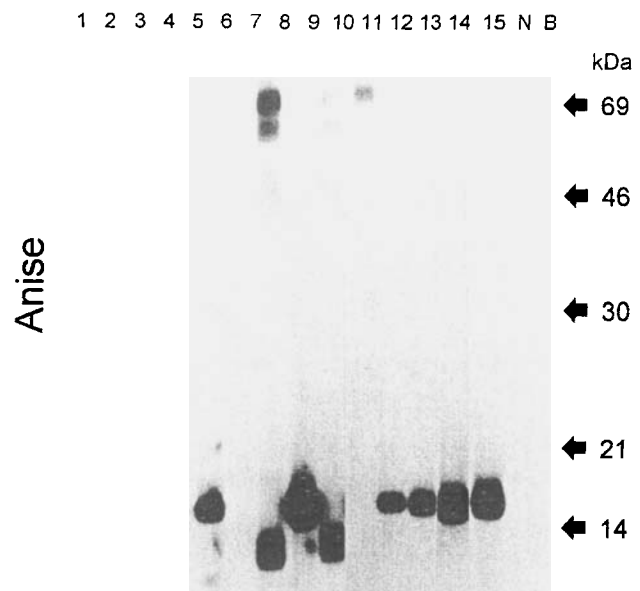


Fig. 1. Typical IgE-binding pattern of Apiaceae spices exemplified by anise. Sera from 15 patients with local and/or systemic reactions to various spices were tested. Characteristically, in about 25–30% of tested sera, no specific IgE antibodies were detectable. Other patients reacted to allergens of 14 kDa (profilin), 17 kDa (Bet v 1 homologues), and IgE-binding proteins with higher molecular weight.

“celery-birch-mugwort-spice syndrome” (5). In a recent study, the allergen composition in different horticultural strains of bell peppers was analyzed (19). By the use of anti-celery-profilin monoclonal antibodies, allergens belonging to the profilin family could be detected in all strains. In contrast to these results, paprika powder – obviously due to the processing procedure – did not contain an IgE-reactive profilin molecule (7). A polyclonal rabbit anti-Bet v 1 antibody recognized Bet v 1 homologues in 4/8 bell-pepper strains. Interestingly, two murine monoclonal anti-Bet v 1 antibodies failed to recognize proteins in these extracts, indicating a narrow spectrum of cross-reacting epitopes. A protein structure with higher molecular weight (between 40 and 60 kDa) again revealed common antigenic structures, obviously abundant in pollens, vegetable foods, and spices (12, 15). In 5/8 bell-pepper extracts, an IgE-binding molecule of 23 kDa could be found. The allergen could also be detected in high quantity in paprika powder (7). N-terminal sequence analysis revealed sequence identity with viroid-inducible, osmotin-like, pathogenesis-related (PR) protein of tomato (20).

From analysis of the different patterns of bell peppers, an interesting conclusion can be drawn: the allergen expression differs from strain to strain (similar observations have been made with other plant-derived food allergens [21]). It can therefore be concluded that plant products with reduced

allergenicity can be produced by breeding. Moreover, this observation supports the concept that transgenic plants with reduced allergen content could be produced; e.g., by antisense or knockout techniques (22). Of course, this strategy cannot be applied when the allergenic gene product has a crucial function in the biology of the plant; in these cases, inactivation of the respective gene would lead to cell death.

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