CASE REPORT

Blackberry (Morus nigra) anaphylaxis

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Background: The blackberry (Morus nigra, Rubus fruticosus) is the fruit of the blackberry bush. There is an important consumption of this fruit in Spain as well as in other countries of the world. Although hypersensitivity to the pollen of the Morus alba tree (Moraceae family) and to its ingested fruit has been reported, no references regarding allergy to the fruit of the blackberry bush have been found in the literature. We report the case of a 45-year-old man who developed systemic anaphylaxis after the ingestion of blackberries. Methods: The sensitization to blackberry and to other fruits of the Rosaceae family was investigated using prick tests and immunoblotting for the detection of specific IgE. Results: The skin tests disclosed hypersensitivity to Morus nigra and to other fruits of the same family. Immunoblotting revealed IgE bands between 25 and 100 kDa, indicating that they were neither lipid transport proteins (which have a molecular weight of about 13 kDa in SDS-PAGE) nor proteins from the Bet v 1 family (16 - 18 kDa). Conclusions: A case of IgE-mediated allergy to blackberry, the fruit of the blackberry bush, is reported. Because of its frequent consumption and of its allergenic potential, this fruit should be taken into consideration in patients with a history of allergy to other fruits of the Rosaceae family and in pollen-sensitised patients with oral syndrome.

Key words: Morus nigra / Morus alba / Rosaceae / Anaphylaxis.

Anafilaxia por moras (Morus nigra)

Fundamento: La mora negra (Morus nigra, Rubus fruticosus) es el fruto de la zarzamora, y su consumo en España y en otros países es importante. Aunque se ha descrito hipersensibilidad al polen del árbol Morus alba (familia Moraceae) y a la ingesta de su fruto, no se han encontrado referencias bibliográficas de alergia al fruto de la zarzamora. Se presenta el caso de un paciente de 45 años que sufrió un cuadro de anafilaxia tras la ingesta de moras. Métodos: Se estudia mediante prick y detección de IgE específica por immunoblotting la sensibilización a la mora y a otros frutos de la familia Rosaceae. Resultados: Se demuestra hipersensibilidad a Morus nigra y a otros frutos de la familia Rosaceae por prueba cutánea. En el immunoblot se detectan bandas fijadoras de IgE entre 25 y 100 kDa, lo que indica que no se trata de proteínas transportadoras de lípidos, cuyo peso molecular oscila alrededor de 13 kDa en la SDS-PAGE, ni de proteínas de la familia del Bet v 1 (16 - 18 kDa). Conclusiones: Se describe hipersensibilidad mediada por IgE a la mora negra, el fruto de la zarzamora. Dado su potencial alergénico y su elevado consumo, hay que tener en cuenta este fruto en pacientes con antecedentes de alergia a otras frutas de la familia Rosaceae y en pacientes sensibilizados a pólones con síndrome oral.

Palabras clave: Morus nigra / Morus alba / Rosaceae / Anafilaxia.
Anaphylaxis (OAS) in relation to fruits and vegetables may affect up to 40% of the pollenosis patients, stressing that this condition is the most frequent clinical form of food allergy in adults. This implies a strong indication for investigating the allergenic potential of fruits and vegetables. Data published by Pastorello et al. confirm a prevalence of the OAS of 26.5% in pollenosis patients. However, fruits may cause food allergy independently of pollenosis as described in Israel, where there are almost no cases of pollenosis but peach is the prime cause of anaphylaxis, and also in Spain, where patients with allergy to pollens of grasses and of the Rosaceae (apple, pear, peach) and patients evidencing only fruit allergy evidence different IgE detection patterns. In the case reported in this paper we tried to demonstrate if the blackberry was able to trigger IgE-mediated sensitization and if this reaction was caused by previously characterised allergens.

CASE REPORT

A 45-year-old male wine merchant residing in a rural area reported having experienced dyspnoea and pharyngeal and labial pruritus over the last 25 years. He clearly related these symptoms to the ingestion of blackberries, raspberries, peanuts and certain brands of margarine, and fruity wines, but most particularly to that of blackberries, with which the oral syndrome was most intense. In the last episode, two months prior to consultation, he developed after the ingestion of two blackberries an oral syndrome accompanied by generalised urticaria, oedema of the glottis, dyspnoea, vomiting, diarrhoea and loss of consciousness. The patient was treated at the Emergency Outpatient Clinic with adrenaline, oxygen, fluid replacement and parenteral corticosteroids, and was discharged after 48 hours.

The allergologic study revealed positive skin tests (prick-by-prick) to blackberry and to a 1/10 w/v blackberry extract (25 x 15 mm). Positive tests were also observed with Chenopodium (6 x 10 mm; specific IgE 10 kU/l), peanut (5 x 5 mm; IgE 1.5 kU/l), wheat (5 x 5 mm, IgE 1.4 kU/l), barley (3 x 2 mm; IgE 1.6 kU/l) and rye (2 x 3 mm, IgE 1.2 kU/l). An oral challenge test with blackberry was not performed because of the severity of the clinical picture and of the patient's refusal.

**In vitro studies.** A blackberry extract was prepared through extraction of the fruits in 10% (w/v) ClNa during one hour at 4°C. After centrifugation (10,000 rpm for 25 min at 4°C) the supernatant was filtered (0.22 µm pore-size filter), glycerinated to 50% and stored at -20°C.

**SDS-PAGE and IgE immunodetection.** The blackberry extract was separated through SDS-PAGE in 10-20% polyacrylamide-tricine gel (Novex, San Diego, CA, USA) under non-reducing conditions, and the separated proteins were electrotransferred to nitrocellulose membranes. After blocking with bovine serum albumin (BSA) in PBS during 2 hours at room temperature, one membrane was incubated overnight with the patient's serum (1:5 dilution) and the other one only with the diluent. The membranes were washed four times with 0.1% Tween 20 in PBS (positive membrane) and another one only with the diluent (negative membrane). The membranes were washed four times with 0.1% Tween 20 in PBS and then incubated for two hours with a monoclonal HE-2 anti-human IgE antibody (1:3000 dilution) and, after renewed washing, with an anti-IgE-peroxidase conjugate (DAKO A/S, Denmark) for one hour (Fig. 2). The detection of components with IgE affinity was quantitated by chemoluminiscence according to the manufacturer's instructions (ECL-Amersham). Sera from patients with no known fruit allergy were used as negative controls (data not shown).
DISCUSSION

The allergens from fruits and vegetables have been investigated with considerable interest in recent years, particularly because of the clinical relevance a possible interrelationship between fruit sensitization and pollinosis may have. Increasing numbers of proteins have been described in relation with the defence mechanisms of plants against the aggression by pests and other pathogens through their antifungal, antiparasitic or antibacterial properties. A very small number of these proteins have been identified as major allergens in vegetable foodstuffs and pollens, with great structural homology. The two best characterised cases probably correspond to the family of pathogenesis-related proteins (PRPs) present in the pollens of *Fagales*, in certain fruits (apple) and in garden vegetables (celery, carrot). The wide distribution of these defence protein families and the high level of sequence similarity between members have been essential for explaining cross reactions between pollens in the first case, and the implication of various grains in the form of occupational allergy known as baker’s asthma in the second one. However, it has been in the course of the last year when suspicions have begun to arise about a close relationship between defence proteins and plant allergens. If confirmed, such a relationship would have important future consequences both in the field of allergy and in the strategies to be used in plant protection, as many transgenic plants base their plague resistance on an overexpression of defence proteins.

In the case here reported no relation appears to exist between a previous pollen sensitization and that to blackberry or to other proteins from the *Rosaceae* family. There are no pollens from *Fagaceae* in the area of residence of our patient, and we suspected in this case, as in others reported from the Mediterranean area, a possible sensitization to the 13 kDa allergens present in a number of fruits in this taxonomic family. It is interesting that, in the previously reported case with *Morus alba* anaphylaxis, the primary sensitization involved the fruit, and the patient later developed hypersensitivity to birch pollen. In the present case, the clinical history appears to be compatible with a primary sensitization to blackberry.

Recent plant allergen characterisation studies, mainly of fruit allergens, appear to implicate three new families of defence proteins, which are ubiquitous and have been well characterised, in allergic reactions: class I chitinases with an N-terminal hevein residue seem to be the major allergens in banana, avocado and chestnut, three of the main foodstuffs associated to the latex-fruit syndrome; lipid transport proteins (LTPs) have been characterised as the proteins with the greatest IgE-binding capability in fruits of the *Rosaceae* family (apple, peach, apricot); the third family is represented by the taumatins (osmotins) detected as allergens in cherries and other fruits.

In the case here reported, we detected IgE specifically directed against 25 - 100 kDa proteins from the blackberry (Fig. 2), which do not appear to belong to any of the previously described defence pro-
tein families. There have been a number of recent reports of allergens specific to the fruits and not shared by the pollens. This has been demonstrated in the case of the peach and kiwi fruit, but there are a number of ongoing studies that will lead to the isolation and characterisation of new allergens from this allergologically very important plant family.

ACKNOWLEDGEMENTS

We gratefully acknowledge the highly professional cooperation of our nursing personnel.

REFERENCES


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