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Rare indoor allergens

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Rare indoor allergens, indoor allergens

Summary
Rare allergens in indoor environment are insufficiently recognized. The sources are diverse: they include animal, namely acaride, insect and mammalian allergens or vegetable allergens. The prevalence of sensitization to rare allergens depends on geographical and climatological characteristics, on people's habits and overall on dwelling specificities. Sensitizations to new rare allergens should be confirmed by documented clinical history, by immunological tests, and by the beneficial effects of avoidance. A review of rare and/or new allergens likely to be present in indoor environment is presented.

Introduction

Individuals are exposed to a wide range of foreign proteins or glycoproteins both in indoor and outdoor environments. Their sources are diverse and include frequent allergens such as house dust mites of the Dermatophagoïdes or Blomia genus, animals and fungal spores. However, a susceptible individual can produce specific IgE antibodies against rare allergens present at home.Such sensitizations depend on the genetic capability as well as on the airborne concentrations of the offending allergens. In this article, we focus on unusual indoor allergens. The arbitrary division between frequent and rare allergens does not necessarily indicate that rare allergens will be clinically insignificant in certain individuals, since their full avoidance may reduce allergy symptoms considerably.

We have excluded occupational allergens, but some of the rare allergens were first described as occupational allergens before they were recognized as major allergens when they were present in dwellings. With the use of monoclonal antibodies, some indoor allergens, first unsuspected, were recognized to be present in homes in significant levels.

Prevalence of sensitization to rare rare allergens depends on geographical and climatological characteristics, house and apartment specificities and persons' habits. Some proteins can act as indoor allergens because of either specific sensitization or special individuals with high allergen reactivity. Ideally, each new rare allergen source should be confirmed by documented clinical presentation, immunological tests, provocation tests and assessment of the beneficial effect of avoidance. Moreover, the proteins corresponding to the allergens in the complex source should be identified

Rare allergens in indoor environment

Acarids

1. Uncommon mites: Houses occasionally have a large

number of storage mites (i.e. Lepidoglyphus destructor, Tyrophagus putrescentiae and longior, Aleuroglyphus ovatus, Gohieria fusca (1-3). The sensitization rate to storage mites has been found high in city dwellers (4, 5). According to Arlian (6), 9.3% of the general population in Ohio (urban, suburban and rural) are sensitized to allergenic products of storage mites (Lepidoglyphus destructor and Tyrophagus putrescentiae). According to this author, surveys in homes should ideally determine the prevalence of allergens and mites of multiple species.

2. Spiders: Only occupational IgE mediated allergy has been reported with spiders; however, as these acarids are frequently brought up in homes as pets, a potential allergy risk may exist. Spiders can provoke rapid vibrations in their bodies, thereby scattering the hairs in the environment (7).
3. Silver fish: It has been demonstrated that house dust contains significant silver fish (Lepisma saccharina) levels. rLep s 1 is the first allergen cloned and characterized from silver fish extract. It is a tropomyosin and has been used to study the importance of the indoor sources of tropomyosin in sensitization (8). However a pathogenic role of silver fish remains to be proved (9).

Insects

1. Cockroaches: Cockroaches are not rare allergens in many countries especially in the warm parts of North America and Asia, but are found infrequently as causes of allergic diseases in Europe (10). The prevalence of cockroach allergy in France, determined by RAST, was less than 5% (11). Moreover, prevalence determined by cutaneous tests or in-vitro methods can be influenced by co-sensitization with other house dust allergens such as mites, which have cross reacting allergens with cockroaches (gluthation transferase and tropomyosin) (12).
2. Among insect allergens, the order of coleopters causes many occupational sensitization in mill workers. In indoor environment, cough and rhino-conjunctivitis exclusively present during house keeping were related to larvae of dermestidae (Attagenus pelio) and the diagnosis was confirmed by cutaneous tests, RAST and IgE determinations to larvae proteins (13). Another clinical case of asthma was reported by Cuesta-Herranz et al (14), induced by dermestidae larvae present in wooden floors, in a dwelling with stuffed animals on the wall. The diagnosis was confirmed by cutaneous tests and specific IgE; moreover a bronchial challenge test induced an immediate response. Environmental control measures were sufficient to control the patient's symptoms (scraping and deinfesting the wooden floor and covering it with a varnish, removing the stuffed animals). Another example is allergic asthma to psocus spp (Pscoptera); these insects have been shown to proliferate in hemp fibers which are used instead of glass-wool fibers for house insulation (15). These reports indicate that it is necessary to be aware of the fact that etiological agents such as, insects present in dwellings, may be important particularly when patients have negative skin test responses to the common indoor allergens.

Other inhalant allergens of insects have been described as outdoor allergens for epidemic asthma, possibly induced by crickets, locusts and moths (Caddis-fly). Some allergies to moths are related to hobbies: for instance, fishing may be a source of exposure to moths and their larvae (16). Fishing hobbyists who are in contact with larvae of chironomids or with their extracts when they feed fishes kept in aquariums reported immediate type hypersensitivity reactions (17, 18). Mairesse (19) reported 7 sensitized subjects (prick-test and specific IgE), among 38 aquarium hobbyists. Four of them suffered from rhinitis and/or asthma and one of them never even fed the fishes. The responsible allergens for sensitizations are haemoglobins of low molecular weight. The main allergens are Chi t 1 and the monomeric component Chi t 1III (20). A cross reactivity with numerous species has been demonstrated especially with IgE binding proteins from Anisakis, German cockroach, Chironomids with several IgE binding components located at 30 to 43 kDa region (21). Other food products such as crustacae or different worms and larvae can also lead to sensitization in fish hobbyists (22). In Japan, higher frequency of IgE antibody responses to insects (moth, butterfly, caddis fly and chironomids) was found in patients with bronchial asthma. Air samplings performed revealed the presence of insect-related particles less than 10 μ in diameter (23). Several cases have been reported recently in the literature describing patients suffering from allergic respiratory symptoms including rhinitis, conjunctivitis and asthma related to Harmonia axyridis exposure (Asian lady beetle, Japanese lady beetle or lady bug) (24, 25).

Any insect growing in large numbers within a house can become a significant source of allergens, this also shows that indoor environments are changing.

Several studies have found IgE antibodies to a wide range of insect species, due to cross reactivities between Der-

matophagoides pteronyssinus, silver fish, coachroach or chironomid, but this does not mean that IgEs antibodies to these insects can be taken as evidence of exposure. The first step towards the suspicion of a potential indoor allergen is to demonstrate its presence at home.

Mammalian allergens

In the indoor environment, household animals are significant sources of allergens. Almost every important mammalian respiratory allergen belongs to the lipocalin family of proteins (12). Outside of cats and dogs, human contact with unusual popular household pets can induce allergic respiratory diseases. Among them, rodents (especially rats and mice) are well known as inducers of occupational respiratory symptoms occurring in laboratory workers (the prevalence of sensitization varying from 14 to 15% (26). However, for other species, the number of exposed persons is unknown and the risk of sensitization is difficult to appreciate. Recently, a large size population survey was performed in Japan using a questionnaire dealing with household conditions including pet keeping and inquiring about respiratory symptoms. In a multivariate logistic regression analysis it appears that there was no association between either dog or cat ownership and respiratory symptoms, in contrast hamster ownership increased the odd ratio for respiratory symptoms (27). Among rodents, hamsters as pets have increased markedly. A clinical report of 30 cases suggests that hamster ownership is associated with mild to severe asthma, sometimes requiring hospital admission and occurring about 15 months after the onset of hamster exposure (28). The search for specific IgE was negative in 8 out of 30 cases. The main allergens differ among different species such as golden hamsters, European hamsters, dwarf Djungarian hamsters (29). Recently, several cases of anaphylaxis after hamster bites have been described (30, 31); a specific allergen from the hamster saliva has been identified. Similar cases have been described after bites by a Mongolian gerbil and prairie dogs (32). Severe asthma symptoms have been described in a patient washing a pet male ferret, specific IgEs were detected especially against urine proteins (33). In an other study of ferret allergy, Immunoblot revealed serum specific IgE binding strongly to a 66 kDa protein of the urine extract suggesting albumin as the relevant épitopes (34). Allergy to mink, a mammal from the same family as ferret has been described in occupational settings (35). Keeping minks as pets can be not unusual in certain countries. The contact to chinchilla in households may lead to sensitization; allergic rhinitis and/or asthma in children and adults have been confirmed by nasal provocation tests in 6 patients (36). Guinea pigs for which the prevalence of symptoms is about 30% in occupational settings can also be kept as pets and induce indoor asthma (37). Guinea pig dusts contain several allergens. These allergens are present mainly in fur, but also in dander, urine and saliva (38). The main allergen isolated from the hair extract is named Cav p 1 (20 kDa) and sensitizes about 70% of patients. IgEs against Cav p 2 (17 kDa) are found in about 55% of sensitized patients. 8% of guinea pig allergic patients exhibit IgE reactivity to serum albumin (39). 40% of guinea pig allergens are carried on small particles (<to 0.8 μ) (40). Rabbits, especially dwarf rabbits are also kept as pets. Among 1602 atopic patients, Liccardi et al. (41) in an Italian multicenter study, found 2.43% rabbit sensitization. Only half of these patients were in permanent or episodic contact with these animals. Only 10% of the sensitized subjects were mono sensitized, they were pet owners and had asthma symptoms. Ory c 1, a 17-18 kDa glycoprotein is found in saliva and in fur (42), Ory c 2 found in several source material and albumin are also rabbit allergens, but of minor importance.

Measuring allergens in settled house dust and in air samplings has shown that the levels of mouse allergens in indoor environments may be similar to those found in animal facilities; mouse allergens were detectable in respectively 80% of dust samples collected in schools (43) and in 100% of bed rooms in inner city homes (44). These recent studies should be completed by the search for sensitization in atopic patients having no occupational exposure in order to evaluate the clinical relevance of mouse allergens as indoor allergens.

Other furry animals, newly introduced as pets, are potential indoor allergens, such as dwarf horses, Vietnam pigs, unusual feline animals, monkeys, or squirrels. Recently a case of domestic allergy to cheetah has been described, confirmed by positive specific IgE to saliva and fur. Inhibition studies and immunoblots showed that besides an homologous allergen to Fel d 1, specific allergens to cheetah are involved (45); this may be explained by the fact that among the Felidae, cheetah and cat belong to different sub-families.

Other animal allergens

Scaly animals such as lizards were assumed not to be allergenic. However, allergy to iguana has been reported and confirmed by skin tests and in vitro studies to iguana scales (46, 47).

Respiratory sensitization to avian allergens has also been described (48). The responsible allergens, especially Gal d

5, an alphalivetin is implicated in the bird egg syndrome (49). More recently, a bird-egg syndrome caused by Agapornis sp. (Lovebirds) has been reported (50). In addition to alpha-livetin the patient developed allergy to sunflower seeds. Severe allergic reactions to sunflower seed and millet have been previously described among bird fanciers (51, 52).

Green algae

Green algae (chlorella) grow under similar conditions to molds and can be found as indoor allergens. Sensitizations to chlorella have been described in children (6% of outpatients in a study from Tiberg (53)) and are mainly found among mold- sensitized patients. The clinical relevance however has not been clearly demonstrated.

Plant derived allergens

The occurrence of allergy due to plant-derived allergens has increased over the past 15 years. These inhalant allergens are found in occupational environments mainly, but they may also be present in the home environment, the prevalence of sensitization to these indoor allergens depending on the number of plants at home. Among ornamental plants, Ficus, especially Ficus benjamina, was found to sensitize 6% of 395 outpatients in Sweden (54) and among them 3% were symptomatic (perennial asthma, rhinitis or conjunctivitis). A lower prevalence of sensitization has been found by Hemmer et al. (55): 2.5% among 2662 atopic patients. Specific ficus allergens were found in house dust samples (56, 57). Allergens are present in latex from ficus, which belongs to the Hevea Brasiliensis family. Other latex plants, such as Euphorbia pulcherina (58) and Araujia sericifera can induce immediate allergies in atopic patients (59). Patients sensitized to ficus have a potential risk of fruit allergy, especially to figs (60, 61). The presence of ficus in hospitals as well as in indoor public places should thus be avoided. In a recent study concerning ornamental plants sensitivity in patients with rhinitis, the most frequent positive prick tests were found with Ficus benjamina followed by yucca, ivy and palm tree (62). Most patients were sensitized to other inhalant allergens and only 13% were sensitized to plants only. Other clinical cases of allergy to ornamental plants have been described as indoor allergens, such as allergy to the coffee plant (63) and papyrus (Cyperus alternifolius) (64). Wütrich and Johansson (61) have reported an allergy case to the ornamental indoor green plant Tradescantia (Albifloxia) (65). Cut and dried flowers are potential allergenic sources, however their incidence as inducers of indoor allergy is a rare occurrence, whereas it is more frequent in gardeners and florists (66-68).

Other vegetable allergens, introduced by human beings, can be present in domestic environment. For instance, powders from Lycopodium clavatum used as dry shampoo (69, 70),or powders such as Fenugrec used as pharmaceutical products in certain ethnic groups (71). This emphasizes the importance of allergens from vegetable origins likely to be present at home and used increasingly for ecological reasons. For instance, pillow padding uses new material such as moth plant (59) and buckwheat, which have been involved in nocturnal asthma (72, 73, 74).

Allergens introduced by stinging and biting

Allergens introduced by stinging can induce allergic manifestations in indoor environment. An example is given by fleas and especially cat fleas as well as by ground bugs (75, 76). European pigeons soft tick Argas reflexus live inside houses and are more and more widespread because of the growing populations of pigeon colonies in urban areas. Bites by ticks usually occur at night and severe allergic reactions are reported (8 out of 12 requiring intensive care) (77). The dominant allergen is Arg r 1, which belongs to the lipocalin family (78); there is no evidence for an increased risk for atopic individuals of developing allergic reactions after an Argas bite (79).

Airborne food allergens

Exposure to airborne food allergens (including handling and cooking) can be induced by odors, fumes, vapors or sprays, which have a potential role in provoking clinical manifestations such as asthma, rhinitis and conjunctivitis in sensitized patients. Reactions induced by peeling vegetables such as raw potatoes, carrots, fresh asparagus are well known (80, 81); but the elicitation of asthma by the steam of cooking vegetables such as chick peas and lentils (82, 83) is also possible. The inhalation of steam when boiling fish or shrimps or other crustacean can also be an inadvertent exposure to allergens in the kitchen. Crespo et al (84) reported 21 children with symptoms of food allergy to fish: 12 among them had rhinitis or asthma after 3 patterns of exposure: fumes generated by frying fish, water vapor released during boiling and mere exposure to fish.

Exposure to airborne allergens, even in low amounts, can induce moderate to severe symptoms in highly sensitized patients. Classical examples are patients with latex allergy who get symptoms in sport settings, patients with mammalian allergy such as allergy to cat or horse, who get symptoms in contact with contaminated clothes brought

inside, or with allergens found in furniture. Patients with peanut allergy may present respiratory symptoms in closed environment such as an airplane cabin where peanut packages are opened. Sicherer et al. (85) reported 62 allergic symptoms occurring during a trip on commercial airliners, with 5 patients needing epinephrine injections. The demonstration of hidden inhaled food allergens (in particular eggs and milk) in indoor environment has been demonstrated by allergen measurements (86, 87).

Conclusion

A wide range of foreign proteins or glycoproteins may be responsible for sensitization in indoor environment. The allergenic content of environment depends on many factors including climatic and geographic variables. It also depends on people's new habits; for instance increasing using of certain plant species in gardening, which can lead to new sources of aeroallergens. One of the most striking points is the emergence of unusual indoor pet allergens related especially to newly-introduced furry animals. Owing to the enthusiasm for ecology, it may be assumed that in the future new unknown indoor allergens may appear. Allergists must know that new sensitizations are to be expected. In order to assess new etiologies, it is essential to document clinical cases by immunological tests; this implies that laboratory support should be available. In any case, the recommendation is to publish the documented clinical cases in order to increase the number of accessible and useful references in the literature, knowing that avoidance of the etiological factors of respiratory symptoms may lead to complete and definitive recovery.

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Allergy to peanut lipid transfer protein (LTP): frequency and cross-reactivity between peanut and peach LTP

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Key words

Food allergy, lipid transfer protein, peanut

Summary

Background: *Lipid transfer protein (LTP) is a widely cross-reacting plant pan-allergen, and sensitized patients may react to many foods. Although peanut allergy is frequently reported by LTP-allergic patients, the evidence of the presence of an allergen homologous to LTP in peanuts is limited.* ***Objective:*** *To assess the prevalence of peanut allergy in patients sensitized to LTP, detect any allergen homologous to LTP in peanuts, and assess its cross-reactivity with peach LTP.* ***Methods:*** *Spanish and Italian adults monosensitized to LTP were interviewed for possible peanut allergy and underwent skin prick tests (SPTs) with peanut extract. Sera from 32 peanut-allergic patients were assayed for peanut-specific IgE by direct ELISA and the Real Test; the serum showing the strongest reactivity was used in immunoblot analysis.* ***Results:*** *74/114 (65%) patients were sensitized to peanuts, and 37 (32% of the whole population; 50% of those sensitized) were clinically allergic. Positive histories were validated by open oral food challenges in 13/13 cases. No SPT-negative patients reported clinical allergy to peanuts. Thus, in this selected population, sensitivity and negative predictive value of peanut SPTs were 100%, whereas specificity and positive predictive value were poor (52% and 32%, respectively). Only 2/32 sera scored positive in both in vitro assays and 4 reacted in the Real Test alone. In immunoblot, the serum studied reacted at about 10 kDa against the peanut extract; pre-adsorption with purified peach LTP totally inhibited such reactivity.* ***Conclusions:*** *Peanut sensitization is frequent among LTP-allergic patients and is clinically significant in about 50% of cases. Peanut tolerance should be assessed in LTP-allergic patients positive on peanut SPTs. Peanut LTP seemingly shares all allergenic determinants with peach LTP.*

Introduction

During the last few years, lipid transfer protein (LTP), the major allergen in the Rosaceae family for patients not sensitized to birch pollen (1-5), has acquired the status of a widely cross-reacting plant pan-allergen (6, 7). Proteins homologous to peach LTP, which is generally considered the most likely primary sensitizer to this allergen, have been detected and characterized in a number of plant-derived foods, including Rosaceae, maize, grape, tree nuts, asparagus, beer, spelt, wheat, orange, lettuce, and cabbage (4,5,8-17). It is now generally accepted that subjects sensitized to LTP may experience allergic reactions following the ingestion of a number of foods and that the likelihood of an allergic reaction to foods which are botanically distant from Rosaceae is directly related to the amount of circulating IgE specific for peach LTP (18). Surprisingly enough, peanuts, one of the foods frequently reported as offending by LTP-allergic patients (6, 7, 18), have not been extensively investigated so far. In a recent international allergy congress (19), hypersensitivity to peanut lipid transfer protein (Ara h 9) was reported, but only a single case report dealing with the clinical significance of peanut LTP, based on ELISA inhibition experiments, has appeared in medical literature (20). The present study aims to assess the prevalence of sensitization and clinical allergy to peanuts among patients sensitized to LTP and to assess the cross-reactivity between peanut and peach LTP.

Patients and methods

Patients

The clinical part of the study was carried out in 4 distinct clinical centers: 1 in Spain (Madrid), and 3 in Italy (Rome, Troina, and Paderno Dugnano). Adult patients monosensitized to LTP seen in the 4 participating centers were included in the study. Monosensitization to LTP was diagnosed in the presence of (a) an unequivocal clinical history of oral allergy syndrome and/or urticaria angioedema and/or anaphylaxis on more than one occasion following the ingestion of peaches, (b) negative skin prick tests (SPTs) with birch pollen extract, and (c) clear-cut positive SPT with a commercial peach extract containing 30 μg/ml of LTP (ALK-Abello, Madrid, Spain). Previous studies showed that this extract lacks both the Bet v 1-homologous allergen, Pru p 1, and profilin (6, 7). Further, although the presence of other unknown allergens cannot totally be ruled out, all patients showing skin reactivity to this extract who were also investigated in vitro (by UniCAP with Pru p 3 or by immunoblot) in other studies reacted to Pru p 3 or to a 10 kDa protein band.

The reasons why peach was chosen as an index food are (a) that this is the fruit most frequently implicated in allergic reactions in patients sensitized to LTP and probably contains the highest amounts of this proteins (18), and (b) that, based on current knowledge, peach lacks other stable cross-reacting plant food allergens including those known to be involved in primary peanut allergy, such as seed storage proteins (legumins, vicilins, and 2S-albumins).

The prevalence of both sensitization and clinical allergy to peanuts was assessed in this population. Patients showing positive SPTs with commercial peanut extract (ALK-Abello 1:20 w/v) but tolerant to peanuts were considered as sensitized, but clinically tolerant. Those showing both positive SPTs and an unequivocal clinical history of peanut allergy were considered as clinically allergic. Italian patients from the latter group were asked to undergo an open oral food challenge (OFC) with peanuts, in order to validate the clinical history (see below). All those who accepted gave an informed written consent before the start of the procedure.

All the patients consented to participate in the study. Since examinations, SPTs, as well as OFCs were carried out as part of the routine diagnostic workup in the 4 participating centers, no Ethical Committee approval was required in Italy. Ethical Committee approval was obtained in Spain.

Twenty-three patients with other types of food allergy (8 shrimp, 5 kiwi, 4 latex-fruit allergy, 2 fish, 2 sunflower seed, 1 tomato, 1 buckwheat) underwent SPTs with the same peanut extract as controls.

Skin tests

Commercial extracts of peach and peanut (both by ALK-Abello) were used to carry out SPTs. SPT were performed on the volar side of the forearm with sterile, disposable 1-mm-tip lancets (ALK-Abello), pricking through a drop of the extract. SPTs with normal saline and histamine at 10 mg/ml were used as negative and positive controls, respectively. Readings were made after 15 min. Reactions were expressed as the mean wheal diameter (adding the longest diameter to the orthogonal diameter and dividing by 2). A mean wheal diameter of 3 mm or more was considered a positive result (21).

In vitro studies
Sera from 32 patients diagnosed as having clinical allergy to peanuts were used in the in vitro part of the study.

Peanut extract - Peanuts were ground in a mixer and then defatted by several passages in diethyl ether. The defatted powder was extracted as a 10 wt/vol suspension in 0.1M phosphate-buffered saline, pH 7.4.
Protein concentration of the extract, measured according to Bradford (22) (BioRad, Milan, Italy), was 10 mg/ml.

Detection of peanut-specific IgE and inhibition studies - IgE specific for peanuts were detected both by direct ELISA, as previously described (23), and by a reverse enzyme allergosorbent test which is not influenced by specific IgG (Real Test, Lofarma, Milan, Italy) (24) using the peanut extract prepared as described above. Both tests were performed at Lofarma Laboratories (Milan, Italy). ELISA and Real Test results were expressed as optical density (OD); based upon the mean value of 4 normal sera (< 400 OD), OD values > 800 were considered positive.
The serum showing the strongest IgE reactivity to peanuts was used in immunoblot analysis.

SDS-PAGE, immunoblot and immunoblot inhibition -
Immunoblot analysis was carried out under reducing conditions. Peanut extract was mixed with LDS sample buffer (Nupage Bis-Tris, Novex, Prodotti Gianni, Milan) and 5% b-mercaptoethanol. The samples were then denatured by heating at 100°C for 5 min. Electrophoresis of extract (25 µg/lane) was carried out in a 10% polyacrilamide precast gel (Nupage Bis-Tris, Novex, Invitrogen, Milan) at 180 mA for 1 h. The resolved proteins were transferred for 1 h onto a nitrocellulose membrane according to Towbin et al. (25). The membrane was saturated with 0.1 mol/l tris-buffered saline containing 5% fat-free milk powder and incubated for 16 h at 4°C with serum (700 µl of serum and 500 µl of saturation buffer). After 3 washings, bound specific IgE was detected by peroxidase-conjugated anti-human IgE antibodies from goat (Biospacific, Emeryville, CA, USA; diluted 1:3500 in saturation buffer) and using an ECL western blotting kit (Amersham, Milan).
In inhibition studies, IgE reactivity was inhibited by preabsorption of the serum with either 10 µg of recombinant peach LTP (26), 60 µg of the peanut extract, or 60 µg of house dust mite extract.

Statistical analysis
In order to assess the clinical usefulness of SPTs with commercial peanut extract in LTP-hypersensitive patients, sensitivity (SE), specificity (SP), positive predictive value (PPV), and negative predictive value (NPV) of SPTs were calculated by established methods (27).

Results

Frequency of peanut allergy among LTP-allergic patients and results of peanut SPTs
The findings in each of the 4 participating centers are shown in Table 1. Out of a total of 114 adult patients monosensitized to LTP, 74 (65%) were positive in SPTs with the peanut extract, and 37 (32%) of the latter reported a convincing clinical history of peanut allergy. Thus, overall 50% of patients sensitized to peanuts (positive SPTs) were peanut allergic. No patient negative in SPTs with peanuts reported clinical allergy to them. These findings were very similar in all the participating centers with the prevalence of peanut sensitization ranging between 53% and 75%, and the prevalence of peanut allergy ranging from 27% to 39%.
Altogether, the SPTs with peanuts showed an excellent SE (100%) and NPV (100%), whereas SP and PPV were poor (52% and 32%, respectively).
No control subjects showed a positive SPT with the peanut extract.

Table 1 - Prevalence of sensitization and clinical allergy to peanuts among patients monosensitized to LTP in the 4 participating centers

Center	No. of patients	No. positive in peanut SPTs (%)	No. with clinical allergy to peanuts (%)
Paderno Dugnano	55	41 (75%)	15 (27%)
Madrid	23	14 (61%)	8 (35%)
Rome/Troina	36	19 (53%)	14 (39%)

Validation of positive clinical histories by open oral food challenges (OFCs)

Of 29 patients with a clinical history of peanut allergy seen in Rome/Troina and Paderno Dugnano, 13 (8 from the Rome/Troina group, 5 from the Paderno Dugnano group) with a history of oral allergy syndrome accepted to undergo confirmation open OFCs with one peanut, and all (100%) experienced an oral allergy syndrome a few minutes after the ingestion. No patient experienced systemic reactions following OFCs.

In vitro studies

In vitro tests were carried out on sera from 32 out of 37 peanut reactors. Only 2/32 patients scored positive on both direct ELISA and the Real Test with the peanut extract, and 4 additional sera showed IgE reactivity to peanuts in the Real Test alone. The remaining 26 sera scored negative in both tests.

In immunoblot analysis (Fig. 1), the serum showing the strongest IgE reactivity to peanuts in ELISA reacted to a protein of about 10 kDa in peanut extract. Such reactivity was totally inhibited if the serum was pre-adsorbed with either purified peach LTP or peanut extract itself, but did not change following pre-adsorption with the house dust mite extract (Fig. 1). A normal control serum did not show any IgE reactivity to peanuts.

In view of the marked differences between the in vivo and in vitro tests, the SDS profiles of the peanut extracts used for the SPTs (ALK-Abello) and for ELISA (Lofarma) were compared. No difference was observed (Fig. 2).

Discussion

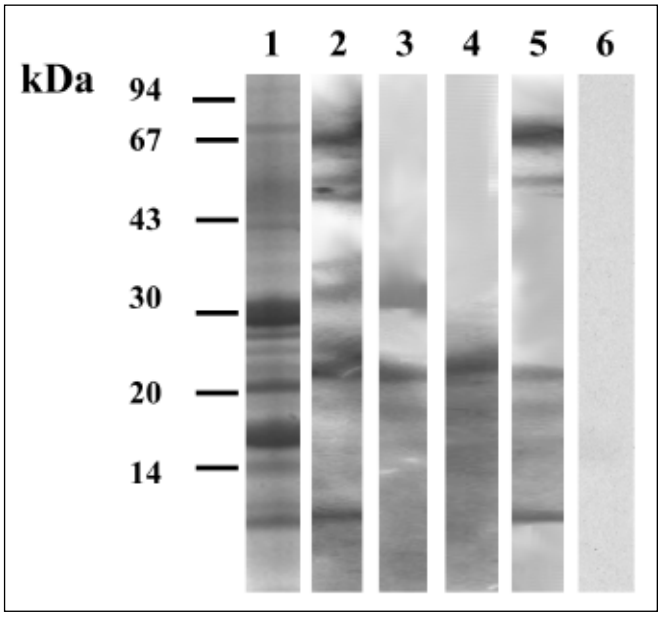
To our knowledge, this is the first study that specifically tries to establish the frequency of both sensitization and clinical allergy to peanuts in patients sensitized to LTP. With the selection criteria adopted, we are confident that patients with both peach and peanut allergy were not sensitized to allergens that have been frequently involved in peanut allergy, namely profilin and Ara h 8, the protein homologous to Bet v 1 (28, 29), but we cannot exclude co-sensitization to other peanut allergens, such as seed storage proteins, although this seems rather unlikely.

More than half of LTP allergic patients from the 4 participating centers showed sensitization to peanuts and comparable percentages had clinical allergy to peanuts, suggesting that despite the geographical differences, the populations studied were homogeneous. These data, which are in line with previous observations (6, 7, 18), suggest that clinical allergy to peanuts occurs in about one third of patients sensitized to LTP. It should be noted that, in this selected population, SPTs with commercial peanut extract showed an excellent NPV, which can be very useful in clinical practice; by contrast, the PPV of SPTs was rather poor, as frequently observed also with different food allergies.

Regarding cross-reactivity between peach and peanut LTP, one study has already provided some evidence using ELISA cross-inhibition experiments (7), while another one found that sera from LTP allergic patients may contain IgE that react to a 10 kDa protein in peanuts (20) and showed cross-reactivity among pomegranate, peanuts,

Figure 1 - SDS-PAGE and immunoblot analysis of peanut extract using the sera from one LTP-allergic patient and from a negative control. Lane 1: SDS-PAGE of peanut extract (25 μg/lane); lane 2: IgE reactivity of the LTP-allergic patient to peanut extract; lane 3: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with recombinant peach LTP (10 μg); lane 4: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with peanut extract (60 μg); lane 5: IgE reactivity of the LTP allergic patient to peanut extract after pre-incubation of serum with mite extract (60 μg); lane 6: IgE reactivity of normal serum on peanut extract.

The allergic patient's serum clearly shows IgE reactivity at about 10 kDa, which is totally inhibited after pre-adsorption with peach LTP or peanut extract, but persists following pre-adsorption with mite extract. The normal control serum does not show any IgE reactivity to peanut extract.

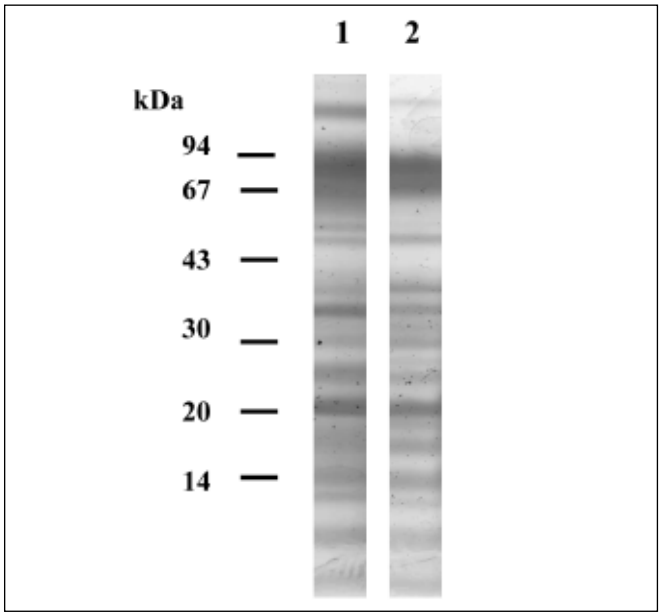


and hazelnuts. In the present study, we have used recombinant peach LTP as an inhibitor and have observed that, in our patient, peach LTP totally inhibited IgE reactivity to peanut LTP in vitro. This finding confirms recent observations showing that recombinant peanut LTP (Ara h 9) strongly cross-reacts with peach LTP (19).

Another aspect that deserves discussion is the much inferior sensitivity of both in vitro methods for detecting specific IgE to peanuts as compared to SPTs in patients sensitized to LTP. Although we did not carry out specific tests in this sense, the presence of low levels of serum specific IgE might be a good reason for this discrepancy, whereas qualitative difference between the extracts used for in vivo and in vitro tests seems rather unlikely, as the SDS-PAGE profiles demonstrate that the two extracts are very similar. The much higher SE of SPTs with respect to in vitro tests has been observed in other food allergies as well (30). It is tempting to speculate that the low sensitivity of in-vitro methods (caused either by the low amount of LTP in peanut, by intrinsic technical difficulties in extracting adequate quantities of this allergen, or by other causes) may be the reason why, despite a rather significant prevalence of clinical allergy to peanuts in LTP allergic patients, so few studies on peanut LTP have appeared in the medical literature, and the only immunological study carried out to date has been performed using recombinant Ara h 9 rather than natural peanut extract (19).

In conclusion, peanut sensitization is frequent among LTP allergic patients, and such sensitization leads to clinical allergy in about half of the cases. In view of the extreme stability of this allergen, which can cause severe systemic allergic reactions, we suggest that clinicians carefully evaluate peanut tolerance in LTP allergic patients positive in SPTs with peanuts. Further, although this is based on the findings with the serum from a single patient, it seems that peanut LTP shares all allergenic determinants with peach LTP, as is the case with all other homologous proteins in fruits and vegetables that have been studied before.

Figure 2 - SDS page of the peanut extracts used for SPTs (left) and ELISA (right). The two extracts show identical profiles.



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Patients monosensitised to Hev b 8 (Hevea brasiliensis latex profilin) may safely undergo major surgery in a normal (non-latex safe) environment

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Key words

Latex allergy, profilin, component resolved diagnosis, allergens

Summary

Background: *Natural rubber latex allergy is a condition at high risk of anaphylaxis during surgery. However, latex contains several protein allergens and not all of them may show the same clinical relevance.* ***Objective:*** *To investigate the clinical relevance of Hev b 8, the natural rubber latex profilin.* ***Methods:*** *Seven patients without a clinical history of latex allergy but identified as being latex hypersensitive by positive SPT (3/7) and or positive latex-specific IgE during routine pre-surgery allergy investigations were studied. All patients were monosensitized to Hev b 8 (Hevea brasiliensis latex profilin) as shown by the detection of specific IgE to recombinant latex allergen components. Ten subjects with a history of latex allergy (urticaria, asthma, and/or rhinitis), sensitised to latex allergens other than profilin were enrolled as controls. Both patients and controls underwent a latex glove-wearing test; in case of a negative test, patients underwent surgery in a normal surgical setting.* ***Results:*** *All 7 patients scored negative on latex glove wearing test and underwent major surgery (orthopaedic, Caesarean section, pilonidal sinus, vascular, tonsillectomy, uterine revision, and uretral surgery) in a normal (non-latex safe) surgical setting without any consequence. In contrast, 9/10 (90%) controls showed a positive latex glove-wearing test ($p < 0.01$).* ***Conclusion:*** *Latex profilin is either clinically irrelevant or is no longer present in latex products. This study highlights the importance of a component-resolved diagnosis of latex sensitisation as a tool to get a more precise assessment of the risk and to reduce the costs of healthcare.*

Introduction

Although less frequent than some years ago (1), IgE-mediated allergy to latex remains a relevant public health problem (2). During the last decade, a number of allergenic latex proteins have been detected and purified (3), and several of them have been found to behave as major allergens. Natural rubber latex (NRL) allergen proteins show differences both in physico-chemical features; this fact may heavily influence the clinical expression of latex sensitisation as well as the cross-reactivity to plant-derived foods. As a consequence, a component-resolved diagnosis of latex allergy may have great clinical usefulness and prognostic relevance. Profilins are well-know pan-allergens in pollen and plant-derived foods (4-7); their importance as airborne allergens is difficult to establish due to the contemporary sensitisation to major pollen allergens, but they have been shown to behave as relevant food allergens (8). The clinical relevance of latex profilin (Hev b 8) (9-11) sensitisation is still unclear. On one hand, patients monosensitised to Hev b 8 score positive on SPT with latex extract and show circulating latex-specific IgE in-vitro as do all other latex-allergic individuals; these findings alarm both the doctors and the patients very much (particularly if the latter have to undergo surgery) due to a potential risk of severe allergic reactions. On the other hand, sensitisation to Hev b 8 is often found in individuals who are undergoing clinical investigation due to respiratory or food allergy but who frequently do not report any problem following latex exposure (12). Although recent studies found that Hev b 8 is present in minimal (if any) amounts in gloves normally used in healthcare settings (12) the final proof of a harmless exposure of such patients to latex gloves during major surgery is still missing. The present study definitively shows that patients monosensitised to Hev b 8 may undergo exposure to NRL material without any consequence.

Patients and methods

Patients

The study was carried out on subjects referred at the allergy department of the XXX Hospital to undergo pre-surgery evaluations because of suspect NRL allergy. The suspect was based on a reasonably suggestive clinical history, on a prior positive SPT with latex extract, and/or on a prior positive latex-specific IgE assay. Several patients had previously undergone surgery in a latex-free environment due to the fear of adverse intra-operative reactions to latex.

Methods

After giving an informed written consent, all subjects underwent SPT with a commercial latex extract (0.016 mg protein/ml; Lofarma Allergeni SpA, Milano, Italy) and measurement of latex-specific IgE levels (ImmunoCAP; Phadia, Uppsala, Sweden). SPT were performed and read following the EAACI guidelines; wheals showing a mean diameter of 3 mm or more were considered positive. Specific IgE values > 0.35 kU/l were considered positive.
Subjects with doubt clinical histories scoring negative on both in-vivo and in-vitro assays were diagnosed as non-allergic to NRL, whereas those positive on SPT and/or ImmunoCAP with or without a clinical history of latex allergy were further investigated by measuring IgE to NRL recombinant allergen proteins (ImmunoCAP; Phadia). Subjects found to be monosensitised to Hev b 8 (latex profilin) represented the "patients" group, whereas those reacting to latex allergens other than Hev b 8 (irrespective of Hev b 8 reactivity) represented the "positive controls".

Glove-wearing test

Both patients and positive controls underwent a latex glove-wearing test. In this test, subjects were asked to wear a latex glove (Sumirubber SDN, Malaysia) on one hand for 15 minutes; the test was considered positive if local itching and erythema/urticaria (with or without angioedema) with or without systemic symptoms (including asthma, and/or urticaria) occurred. The test was immediately stopped if systemic symptoms developed. Five normal subjects underwent a latex glove-wearing test using gloves of the same lot of those used for both patients and positive controls. The latex glove-wearing test was carried out and personally read by a physician (13)

Surgical treatments

Latex-reactive subjects with both negative clinical history and negative latex glove-wearing test underwent their respective surgical treatments in a normal hospital setting (i.e. using latex gloves, catheters, endotracheal tubes, etc).

Those with a positive clinical history and/or a positive latex glove-wearing test underwent surgery in a latex-free environment.

Statistics

Proportions were compared by the chi-square test with Yates' correction. Means were compared by two-tailed Student's t test. Probability values < 5% were considered statistically significant.

Results

Seven patients monosensitized to Hev b 8 (M/F ratio 3/4; mean age 27.1 years, range 14-46 years) (table 1), and 10 positive controls (M/F ratio 3/7; mean age 28.7 years, range 10-38 years) (table 2) were studied. The two groups did not differ significantly in mean latex-specific IgE levels. In contrast, 0/7 (0%) patients vs 9/10 (90%) controls showed a positive latex glove-wearing test ($p < 0.01$). The glove-wearing test was negative in 5/5 normal subjects.

Since no patient had a history of latex allergy, all 7 underwent their respective surgical treatments in a normal hospital setting without any adverse consequence (see below). In contrast, in the light of the positive clinical histories, of specific IgE findings, and of positive latex glove wearing test, all control subjects underwent surgery in a latex-free environment.

Patients case reports

A 17-year-old girl with a long-lasting history of seasonal rhino-conjunctivitis and asthma associated with multiple pollen sensitisation (birch, grass, weeds) and sensitisation to a number of plant derived foods (including tomato, Apiaceae, Rosaceae, potato, kiwi, melon, avocado, and tree nuts) had to undergo orthopaedic surgery due to ankle fracture. SPT with latex extract scored strongly positive (mean wheal diameter 12 mm), although a history of immediate allergic reactions following contact with latex goods was missing. Measurement of serum specific IgE to various recombinant latex allergen proteins showed significant reactivity to profilin (Hev b 8) and only a weak reactivity to Hev b 6 and Hev b 11 (table 1).

A 46-year old pregnant woman with a history of multiple pollen allergy and oral allergy syndrome following the ingestion of a number of raw plant-derived foods was evaluated before delivery. SPT with commercial latex extract scored strongly positive (mean wheal diameter 15 mm) in spite of a negative history of latex allergy. In-vitro tests showed single IgE reactivity to latex profilin. Since the latex glove-wearing test did not induce any appreciable reaction Caesarean section was carried out in a normal surgical setting without any consequence.

A 34-year old man underwent allergy evaluation before pilonidal sinus surgery. He had a history of both birch and grass seasonal rhino-conjunctivitis and of oral allergy syn-

Table 1 - Levels of IgE specific for latex allergen proteins, grass pollen profilin, and birch pollen profilin in 7 cases.

Patient	1	2	3	4	5	6	7
Sex/age	F/17	F/46	M/34	M/38	M/14	F/27	F/14
Glove wearing test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Latex Extract	2,43	1,15	5,73	1,14	3,22	1,97	1,14
rHev b 1	<0,10	<0,10	<0,10	<0,10	0,14	<0,10	<0,10
rHev b 3	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 5	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 6.01	0,79	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 6.02	0,77	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 8	4,33	2,29	5,21	0,51	2,51	5,33	0,51
rHev b 9	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 11	0,60	<0,10	<0,10	<0,10	0,14	<0,10	<0,10
rPhl p 12	3,71	0,97	2,08	1,22	2,88	17,4	1,22
rBet v 2	3,37	0,92	2,90	13,5	1,60	2,97	13,5

Values are in KU/L

drome following the ingestion of several fresh plant-derived foods. The man did not report a history of latex allergy but showed clear-cut positive SPT with latex extract (mean wheal diameter 10 mm). In-vitro analysis demonstrated monosensitivity to Hev b 8. Since the latex glove-wearing test did not induce any clinical response, the patient underwent surgical intervention in a normal hospital setting.
Four further patients, a 38 year-old man, a 14 year-old boy, a 27 year-old woman, and a 14 year-old girl, all with a history of both grass and birch pollen allergy and of oral allergy syndrome following the ingestion of a number of plant-derived foods were found to have circulating latex-specific IgE (1,14 KU/l, 3,22 KU/l, 1,97 KU/l, and 2,60 KU/l, respectively), in spite of a negative SPT with latex extract. In-vitro analysis showed monosensitivity to Hev b 8 in all four cases. After a latex glove-wearing test was carried out, in all cases with negative results, these patients underwent vascular surgery, tonsillectomy, uterine revision, and uretral surgery, respectively, in a normal setting.

Positive controls (table 2)

A history of urticaria, rhinitis, and/or asthma upon contact or inhalation of latex was present in 8, 7, and 5 cases, respectively. Three of them had a history of latex-fruit allergy syndrome (offending foods avocado [n=2], chestnut [n=2], peach, banana and kiwi m[n=1]). All these patients scored positive on SPT with latex extract. No patient showed IgE reactivity to Hev b 8; 9 patients reacted to Hev b 6, 3 to Hev b 5 (1 monosensitive), 3 to Hev b 11, and 1 to Hev b 1.

Discussion

All our Hev b 8-monosensitized patients underwent general surgery in a normal (not latex-free or latex-safe) setting without any problem. As shown by component-resolved diagnosis in-vitro, all of them, but one that showed a weak additional reactivity to Hev b 5 and Hev b 6, were sensitised uniquely to latex profilin as a consequence of primary pollen sensitisation. Although the number of patients included in this study is limited due to the difficulty in recruiting patients that are monosensitized to latex profilin and have to undergo surgery, our observations suggest that single sensitisation to Hev b 8 is unlikely to result in allergic reaction upon exposure to latex and does not represent an indication to a latex safe medical/surgical practice. Whether this is the consequence of the lack of profilin allergen in latex devices (12,14) or of a clinical irrelevance of the allergen per-se (8) has to be established.

Table 2 - Levels of IgE specific for latex allergen proteins in 10 positive controls

Control	1	2	3	4	5	6	7	8	9	10
Sex/age	F/33	F/33	F/16	M/33	F/37	M/16	F/38	F/33	M/10	F/38
Glove wearing test	Positive	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Latex Extract	44.4	1,97	1.95	0.5	5.8	0.8	25.8	3.7	32	1.03
rHev b 1	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	0,14	1,5	<0,10
rHev b 3	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 5	8,9	<0,10	2,2	<0,10	<0,10	<0,10	18,1	<0,10	4,4	0,10
rHev b 6.01	16,7	0,91	<0,10	0,9	6,9	0,7	9,4	3,9	49,5	1,2
rHev b 6.02	8.8	0.94	<0,10	1.0	7.2	1.0	8.8	5.3	48.5	1,5
rHev b 8	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 9	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10	<0,10
rHev b 11	0.2	0.27	<0,10	<0,10	2.1	<0,10	1.6	<0,10	0.3	<0,10
rPhl p 1		25.0				34,1				1,96
rPh p 12		1,0				0,1				0,1
rBet v 1	22,1						12,5			
rBet v 2	0,1						0,1			

Values are in KU/L

Until recently, clinical decisions regarding latex-hypersensitive subjects to be submitted to surgical treatments had to be based on the measurement of total IgE and of latex-specific IgE levels and on questionnaires (15, 16). However, our study suggests that latex-specific IgE levels cannot be adopted as a reliable means to discriminate between patients at high or low risk of adverse reaction upon contact with latex, as shown by the latex glove-wearing test. Even this latter procedure, although useful in detecting patients likely to react upon latex contact, does not seem totally reliable, as it scored negative in 1 control subjects with a history of latex allergy and specific IgE levels for rHev b 6. In effect the usefulness of the "use test" has been questioned in view of the widely varying allergen contents of gloves from different manufacturers and from different lots (17).

On the other hand, one patient showing sensitization to profilin and a weak additional reactivity to Hev b 5 and Hev b 6 showed a negative provocation test and underwent surgery in a normal setting without any consequence, suggesting that such additional IgE reactivity was clinically irrelevant although this needs to be established by a proper follow-up program. It is also possible that the recent improvements in manufacturing processes resulting in an overall reduction of latex allergens levels in surgical gloves may have played a role in the negative latex glove wearing test as well as in the absence of any intra-surgery allergic reactions in this patient (18).

In conclusion our study provide evidences that component-resolved diagnosis is a more sensitive marker than latex specific IgE for the outcome intra-operatory anaphylaxis in patients sensitised to latex who undergo surgery. It may also help clinicians to take decisions that may eventually reduce the costs of healthcare (e.g. avoiding unnecessary latex-free procedures) without any increase in risks.

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Immediate hypersensitivity to penicillins with negative skin tests – the value of specific IgE

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Key words

Allergy, penicillins, hypersensitivity reactions, predictive value, specific IgE, skin testing

Summary

The determination of specific IgE in patients with history of penicillins hypersensitivity is simple, safe and widely available. The positive and negative predictive values of this determination, however, are not yet established. In order to evaluate them, we performed specific IgE determination and diagnostic drug challenges in a group of 22 patients with a clear history of immediate penicillins hypersensitivity but negative skin tests. In this sample, the positive and negative predictive values were 29% and 87%, respectively. This seems to indicate that a positive specific IgE is not enough to confirm the diagnosis, and further study is necessary.

Beta-lactams are the most commonly used antibiotics, accounting for 2/3 of those available on the market, and the most frequent cause of antibiotic hypersensitivity reactions. Among them, natural and semisynthetic penicillins are responsible for more than 75% of those episodes (1). Hypersensitivity reactions are a major health concern as they can be a significant cause of morbidity and mortality, limit therapeutic options and consequently alter the pattern of microbial resistances and increase socio-economic costs (2). For these reasons, the study of all suspected cases of penicillins hypersensitivity is highly important, to avoid the unnecessary use of less efficient or more expensive alternatives, due to fear of a reaction.

Currently, a firm diagnosis is based on a detailed clinical history, skin testing (prick and intradermal tests), specific IgE determination and drug challenge when the previous are both negative and the reaction is not life-threatening (3,4).

Several previous studies have focused in determining the sensitivity, specificity, positive and negative predictive value of skin tests for diagnosis of penicillins hypersensitivity reactions (5,6). The determination of specific IgE in patients with history of penicillins hypersensitivity is a simple, safe and widely available tool. To our knowledge, however, there has been no study concerning predictive values of specific IgE determination. This is of utmost importance in order to determine if a positive IgE in patients with history of penicillins hypersensitivity reaction makes drug challenge tests unnecessary. In a study by Blanca *et al* (7), 42% of 26 patients with negative skin tests and a positive drug challenge had positive specific IgE to benzylpenicilloyl or amoxicilloyl, suggesting that this subgroup of patients could have been diagnosed by specific IgE determination alone, obviating the need to challenge them. But this still does not answer the ques-

tion whether all patients with positive specific IgE are truly allergic without doing a drug challenge. Particularly troublesome are the cases with negative skin tests and positive specific IgE: is it a true or false-positive result? Another important question relates to patients with a clear hypersensitivity history but with both negative skin tests and specific IgE: can we be sure they really are not allergic without doing a provocation test?

Following a previous paper (8) about the diagnostic work-up in patients with history of beta-lactam hypersensitivity reactions, and to determine the positive and negative predictive values of specific IgE in patients with negative skin tests (prick and intradermal), we performed specific IgE determination and diagnostic drug challenge tests in a group of 22 patients (8 male, 14 female), with 40.4 ± 19.0 years of age and a clear history of penicillins hypersensitivity reactions but negative skin tests to PPL and MDM (or penicillin), amoxicillin and ampicillin. All patients had history of an immediate reaction, with a cutaneous presentation in 13 cases, respiratory in 3, cutaneous+respiratory in 4 and gastrointestinal in 2. The implicated antibiotics were penicillin and amoxicillin, with 11 cases each. Cut-off for IgE was considered 0.35 kU/L, with values above or equal this considered positive and those below it considered negative (UniCAP-System®, Phadia, Uppsala, Sweden). Out of the 22 tested patients, 7 had positive specific IgE to penicillin G or V, amoxicillin or ampicillin, with values ranging from 0.40 to 2.90 kU/L and the remaining 15 had negative specific IgE to the same antibiotics. In patients with positive IgE, the elapsed time between the IgE measurement and the drug challenge was, on average, 22 months (ranging from 6 to 41 months) and none of the previous reaction had been life-threatening. The drug challenge tests were done according to ENDA guidelines (4), under strict medical surveillance. Increasing doses (four or five), of the antibiotic to which the patients had positive specific IgE were administered each 30 min until the therapeutic dose was achieved. The symptoms and signs were monitored during the challenge, as well as pulse, blood pressure and PEF measurements. In order to evaluate non immediate reactions, patients stayed under medical surveillance for 3 hours after finishing the challenge and were instructed what to do if any reaction occurred after being dismissed from the hospital.

Of the 7 tested cases with positive specific IgE, only 2 had a positive reaction to the drug challenge, both immediate and with cutaneous involvement (similar to the previous reaction). The remaining 5 patients had no immediate nor late symptoms.

Of the 15 tested cases with negative specific IgE, 13 had no immediate nor late symptoms and only 2 had a positive reaction to the drug challenge, one of them immediate and the other delayed, both mild and non life-threatening.

Despite being a very small group of patients and keeping in mind the wide interval between in vitro IgE measurement and drug provocation test, it seems reasonable to question the positive predictive value of specific IgE in the diagnosis of penicillins hypersensitivity reactions since in our sample it was only of 29% (2 out of 7 patients). This seems to indicate that a positive specific IgE in patients with history of penicillins hypersensitivity but negative skin tests is not enough for confirming the diagnosis, and further study (challenge test) is mandatory. In a study by Petersen *et al* (9), the CAP-FEIA (Phadia) system showed that despite being highly sensitive, it is susceptible to false-positive results due to irrelevant specific IgE antibodies with low affinity and also to cross-reacting IgE. This may be the case in our study. However, we cannot exclude the hypothesis that a decrease in specific IgE levels over time, as has already been demonstrated (3,10), as an explanation for the low positive predictive value of positive specific IgE in these patients.

In contrast with the positive predictive value, the negative predictive value of specific IgE in patients with history of penicillins hypersensitivity but negative skin tests is much higher (13 out of 15 patients, 87%), and is probably enough to confirm the diagnosis of IgE-mediated allergy.

More studies, with a larger number of patients and smaller time intervals between the IgE determination and drug challenge are needed to establish the real positive predictive value of positive specific IgE in patients with negative skin tests.

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Anaphylactic reaction to hydroxyzine in an anesthetized patient

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Key words

hydroxyzine, anaphylaxis, anesthesia, premedication, perioperative allergy

Summary

A case of anaphylaxis occurring during a general anesthesia is presented. The reaction was severe with bronchospasm and hypotension (grade 2 in the severity of per-operative anaphylactic shock). The responsibility of hydroxyzine, administered for premedication was suspected by intradermal testing with the molecule, which was twice positive at a 10^{-2} dilution of the commercial solution. The same test remained negative in 5 control subjects. All the other drugs received during anesthesia gave negative results. Using the same protocol excepted for the use of hydroxyzine a new general anesthesia could be performed under a premedication with dexchlorpheniramine without any allergic reaction. Anaphylactic reactions are very rare with hydroxyzine used in premedication for anesthesia in regard to the large prescription of the drug. Only two previous cases were reported but attention of the allergist must be also pointed towards the medications received in the perioperative period as for the anesthetic drugs

Introduction

Histamine release may induce life-threatening side effects associated with drugs as anesthetics, antibiotics or contrast media. While the mechanism of release during an anesthesia is mainly immunologic, a part of the reactions may be prevented by the use of histamine receptor antagonists (1). Hydroxyzine hydrochloride is a histamine H1 receptor antagonist that is effective in the treatment of chronic urticaria, dermatitis, and histamine-mediated pruritus. As it has also sedative properties, it is a premedication widely used before an anesthesia in allergic patients (1), administered orally or intravenously.

Hypersensitivity reactions to hydroxyzine are sparse, mainly of delayed mechanism with cutaneous signs (2-8). We want to report the case of a patient who presented an anaphylactic reaction related to hydroxyzine during an anesthesia. We described an alternative premedication proposed to the patient for further anesthesia.

Case report

A 60 yr old woman was scheduled for thyroidectomy because of toxic nodules. Her past history revealed an uneventful left thyroidectomy 25 yr ago. In her medical his-

tory it was noted an arterial hypertension, a dyslipidemia, no respiratory problem. She was not atopic, but reported skin rashes or pruritus with paracetamol, aspirin, codeine, some antihistamines and many anti-inflammatory drugs. She took daily losartan, levothyroxine, fenofibrate, and lorazepam at evening.

Just before surgery she received 100 mg of i.v. hydroxyzine plus 80 mg of methylprednisolone. General anesthesia was induced by i.v. propofol, midazolam and sufentanil without the use of curare. A tube was easily inserted in the trachea and anesthesia was maintained by sevoflurane. However, 10 minutes after i.v. injection of anesthetics, she experienced a bronchospasm with an increasing in airway pressures associated with mild hypercarbia at 55 mmHg and a decrease in arterial blood pressure from 130/80 to 90/50 mmHg. The intensity of bronchospasm decreased with terbutaline and budesonide spray plus 40 mg of i.v. methylprednisolone. Surgical procedure started by preparing the skin with alcoolhic povidone. Neverveless, a few minutes after, a new episode of bronchospasm occurred with a drop in pulse oxymetry at 0.90 despite the use of FiO2 1. Surgery was cancelled. The patient received 0.25 mg and continuous infusion of i.v. terbutaline. She was admitted in the post-anesthetic care unit (PACU). Thirty minutes after the onset of the bronchospasm, blood was sampled to determine the concentrations of histamine and tryptase. As respiratory function remained stable, tracheal tube was removed 20 minutes later. Terbutaline administration was stopped in the evening. In operative room and PACU no cutaneous signs were observed. Three months later, the patient was addressed to our allergy clinic to diagnose the reaction and propose an alternative premedication for further anesthesia.

The allergologic check-up was assessed with blood chemistries, cutaneous tests and provocative reintroduction test.

At time of the anaphylactic reaction, tryptase level was at 80 mcg.L^{-1} (N < 13 mcg.L^{-1}), and histamine at 150 nM.L^{-1} (N <10 nM.L^{-1}).

At time of the allergologic assessment, the pulmonary function was normal and the provocative test to metacholine was negative with a Pd 20 > 2000 mcg. Basal level of tryptase was 3 mcg.L^{-1}, and histamine 11 nM.L^{-1} discarding any pathology as mastocytosis. Total IgE level was considered as normal at 36 U.mL^{-1} (N <100) and specific IgE against latex were negative in CapRAST^{R}.

Skin tests were performed in accordance with drug allergy European Network of Drug Allergy/European Academy of Allergology and Clinical Immunology recommendations (9) .The cutaneous reactivity was important to codeine and histamine controls, and negative for saline solution. The cutaneous prick and intradermal testing showed negative responses for latex, midazolam, propofol and sufentanil. The skin prick-tests (SPTs) were also negative to hydroxyzine (dilution10^{-1} of the commercial solution), methylprednisolone, and povidone (under several galenic forms). Intradermal tests (IDTs) were negative excepted for hydroxyzine which induced a wheal of 14 mm when an injection of 0.05 ml at 10^{-2} dilution of the commercial solution (100mg in 2 mL) was performed.

In order to propose an alternative premedication for further anesthesia, a new screening by cutaneous tests and provocative reintroduction tests was performed several months later. The skin tests were identical, with a wheal of 15 mm after hydroxyzine (at 10^{-2}), contraindicating a reintroduction challenge test with hydroxyzine. The skin tests (SPTs in native form for all drugs, IDTs from 10^{-3} to 10^{-1} of the commercial solution for disposable soluble forms) with several antihistaminic agents (cetirizine, dexchlorpheniramine, ebastine, loratadine and desloratadine, mequitazine) were negative. So we choose to perform an oral reintroduction test with dexchlorpheniramine. Finally, a total dose of 6.6 mg of dexchlorpheniramine (step by step 4.1 mg orally and 2.5 mg intramuscularly) was given without any immediate or delayed adverse reaction.

Under 2 mg of dexchlorpheniramine and 0.25 mg of alprazolam premedications given orally before thyroidectomy, general anesthesia with propofol, sufentanil and sevoflurane was uneventfully performed. The follow-up of our patient during 3 days was simple without any allergic reaction.

Discussion

There are only few case reports quoted in the Medline database, and hypersensitivity reactions with histamine receptor antagonists are mostly of delayed type (2-8). The signs reported with hydroxyzine are skin rashes, urticaria or photosensibilisation, erythema multiform with positive patch tests, fixed drug eruption or systemic eczema. There are only 2 case reports of hypersensitivity reactions to hydroxyzine during an anesthesia (10,11). In the first case hypoxemia and skin eruption were noticed during an orthopedic procedure (10), and in the second a generalized urticaria occurred 30 minutes after hydroxyzine premedication during cardiac surgery (11). In the former case skin tests were positive to hydroxyzine, in the second case

an immunological mechanism has been evoked because lymphocyte stimulation test turned positive to hydroxyzine. In our patient, a severe bonchospasm associated to an arterial hypotension occurred after induction of anesthesia. The high concentrations of tryptase and histamine after the reaction and the positivity of intradermal tests (positive twice) supported the diagnosis of an immediate hypersensitivity reaction to hydroxyzine.

As positive skin tests with hydroxyzine have not been yet reported, we performed the same tests to 5 control subjects. They remained all negative in IDTs up to a 10^{-1} concentration of the commercial solution. In order to rule out the responsibility of compounds found in hydroxyzine tablets, which are also contained in surgical antiseptic distemper, we tested povidone in SPTs, which were all negative in our patient.

This is the first case of a well documented immediate hypersensitivity reaction to hydroxyzine. Mostly curares, latex and antibiotics are responsible for such events during an anesthesia (12). The anaphylactic reaction to hydroxyzine was annoying because such agent is often given to neutralize the effects of histamine release after administration of anesthetics, and histamine receptor antagonists are the premedication of choice in drug sensitive patients. As our patient must be again anesthetized, it was necessary to find an alternative to hydroxyzine as premedication. Among the histamine receptor antagonists, cetirizine and hydroxyzine share a common core, the piperazine core. We speculated that epitope for hypersensitivity reaction in our patient was perhaps the piperazine core (3,5,6,13) because the patient reported previous rashes with cetirizine. However, SPT remained negative with cetirizine but also with hydroxyzine certainly due to a lower sensitivity of SPT compared to IDTs (9)

Cetirizine was not suitable for IDTs so we could not conclude about a sensitization to the drug without a provocative test which was not performed for ethical reasons.

Nevertheless, to find an alternative to hydroxyzine as premedication before an anesthesia, we decided to perform provocative test with an other compound family rather than with cetirizine in such indication. In regard to its sedative properties and considering a possible iv administration, we test dexchlorpheniramine by using IDTs and oral and systemic reintroduction tests. As skin tests and provocative challenge were negative we proposed it as premedication before the new anesthesia, which was uneventful.

In conclusion, we report a per-operative anaphylaxis to hydroxyzine used in premedication before an anesthesia. The allergological investigation supports the hypothesis that it may be considered as a potential allergen for immediate hypersensitivity reaction. By using screening tests and provocative reintroduction test we found an alternative to hydroxyzine to prepare the patient before the new anesthesia. Moreover, our case report underlines that it is important to test also the molecules used for premedication in the diagnosis of immediate hypersensitivity reactions occurring during an anesthesia.

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Gluten-free food as source of hidden allergen (lupine)

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Key words

Food allergen, lupine, gluten-free food

Summary

A woman, 68 yrs, developed an anaphylactic reaction after tasting a few pieces of gluten-free pasta. She was not celiac but was preparing a meal for her celiac nephew. The culprit pasta contained lupine flour and lupine proteins. Prick test with lupine extract was positive. ELISA and immunoblot analysis showed the presence of specific IgE to lupine in patient's serum.

Lupine allergy was first described in 1994 (1). Since then many cases of lupine allergy have been described, probably because the inclusion of lupine flour in food has steadily increased during the last decade. Allergic sensitization to lupine is considered clinically relevant especially in peanut-sensitized individuals, both adults (2) and children (3), although some cases of primary lupine allergy have also been described (4-6). This report describes an adult, without a history of food allergy or of sensitization to peanut, who developed an immediate systemic allergic reaction (anaphylaxis) after eating a few pieces of gluten-free pasta made with lupine flour.

Case report

A 68 year-old woman was referred to our service for allergological evaluation after an episode of generalized urticaria, epigastric pain, ocular itching, periocular oedema and dyspnoea occurring about 30 minutes after tasting 3-4 small pieces of gluten-free pasta ("maccheroncini") while preparing a meal for her celiac nephew.
The allergic reaction was promptly treated at the E.R. of the local hospital and there were no subsequent reactions.
Personal history was unremarkable except for the presence of seasonal rhino-conjunctivitis since the age of 40 yrs.
The label of the culprit pasta (BiAglut PastaMia®) declared the following ingredients: maize starch, potato flour, lupine flour and lupine proteins, fat acids.
SPT with commercial food extracts (cereal mix, legume mix, peanut, soy, peach, tomato, walnut, hazelnut, spices mix, cod, milk, white egg, yolk, almond, potato, shrimp, mussel) and pollens (grass, mugwort, pellitory, birch, hazelnut) (Lofarma S.p.A., Milan, Italy) were all negative except for birch pollen (mean diameter of the wheal 8 mm). SPT with lupine extract was positive (mean diameter of the wheal 10 mm). Histamine 10 mg/ml (positive control) 6 mm.

Patient's serum was positive to lupine extract as assessed by an ELISA IgE assay: 1,7 vs. 0,3 (control serum).
Immunoblot analysis showed that a certain number of components were recognized by patient's serum. More specifically, a wide zone comprised between about 50-100 kDa and a more restricted zone at about 18 kDa, perhaps corresponding to 2S albumins (Figure 1).

Discussion

The patient, according to the results of skin testing, was sensitized to both birch pollen and lupine, but not sensitized to peanuts. ELISA IgE assay and immunoblotting confirmed the presence of specific IgE to lupine in patient's serum. A cross-reactivity between birch pollen and lupine due to a Bet v 1 homolog allergen seems unlikely since Bet v 1 homolog allergens are heat- and pepsin-labile while the patient developed the anaphylactic reaction after the ingestion of pasta boiled at 100 °C for several minutes. Moreover, the patient, although sensitized to birch pollen, has never shown an oral allergic syndrome after the ingestion of fresh fruits (for example apple) which are a well known source of Bet v 1 homolog allergens. A recent study has shown that lupine allergy is more complicated than previously thought because many allergens are involved, both cross-reactive with other legumes and unique for lupine (5). Our immunoblot analysis showed a pattern of multiple recognition by patient's serum. The clinical pattern of the reaction lends support to the hypothesis that a stable allergen, not cross-reacting with peanut or other legumes, was primarily involved.
This case shows that gluten-free foods can be a source of hidden allergens and that their consumption is not exempt from allergological risks. Moreover, since celiac disease and IgE mediated allergy are independent phenomena that can coexist in the same individual, the repeated ingestion of lupine flour in celiac subjects using gluten-free food could be a potential risk for allergic sensitization; further studies are needed to elucidate this point.

Appendix

Preparation of lupine extract

Eight grams of defatted lupine flour was submitted overnight to an aqueous extraction in 100 ml of 0.1M phosphate-buffered saline, pH 7.4 (PBS).

After centrifugation supernatant was harvested and dialyzed against saline by membrane at 3.5 cut off, before to be filtered through a 0.22 μm membrane. Protein content was determined by Bradford's method and resulted 8.2 mg/ml. For SPT preparation, lupine extract was diluted 1:2 with glycerin.
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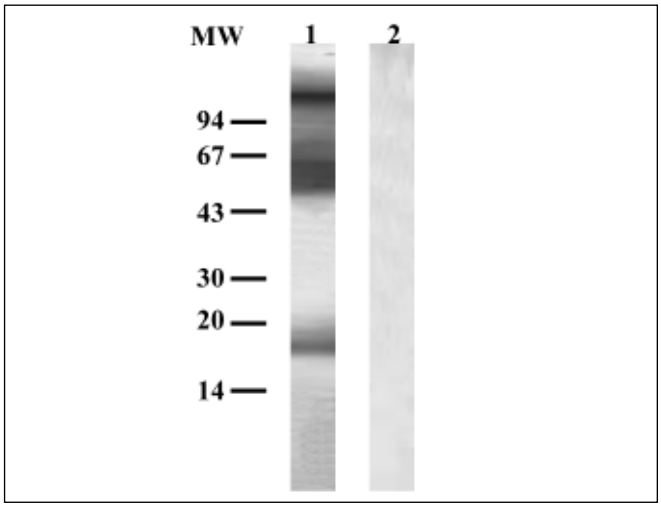
ELISA IgE

Two μg/100 ul (coating buffer: 15 mmol/L Na2CO3 and 35 mmol/L NaHCO3, pH 9.6) of lupine extract, (Maxisorp Nunc, Roskilde, Denmark) were added to 96-microtitre wells for coating phase. After washings, wells were saturated with 2% bovine serum albumin (BSA) in PBS (dilution buffer) for 2 hours at r.t.. Subsequently 100 ul of sera from normal subject and patient were added to wells and incubated for 2 hours at r.t.. Specific IgE was detected by adding a peroxidase-conjugated anti-human IgE goat serum (diluted 1:3500, Biospacific, Emeryville, CA, USA); a colorimetric reaction was induced by using tetramethylbenzidine/H2O2 as substrate. The enzyme reaction was stopped after 20 minutes by the addition of 1 mol/L HCl. Absorbance values (O.D) were read at 450 nm by spectrophotometer. Serum was considered positive when its OD value is at least two times higher than control one.

Figure 1 - IgE reactivity on lupine extract of patient's serum (lane 1) and normal serum (lane 2). M.W.: molecular weight standards



Immunoblotting

Electrophoresis of lupine extract (12 μg per lane) was carried out in a 10% polyacrilamide precast Nupage Bis-Tris gel according to manufacturer instructions (Invitrogen, Milan, Italy) at 180 mA for 1 h. The resolved proteins were transferred onto a nitrocellulose membrane (Protran BA 85, Schleicher and Schuell, Milan, Italy) according to Towbin (7). The membrane was saturated in TBS buffer containing 5% defatted dry milk (saturating buffer) and incubated with patient's serum or control normal serum diluted 1:2 in saturating buffer. Bound specific-IgE were detected by adding of peroxidase-conjugated anti-human IgE goat serum (diluited 1:1500, Biospacific, Emeryville, CA, USA) and ECL western blotting kit (Amersham, Milan, Italy) as substrate.

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Allergic reactions during allergy skin testing with food allergens

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Key words

Food allergy, meat allergy, fish allergy, Skin prick tests, Prick-to-Prick tests, Anaphylaxis

Summary

Skin testing is a reliable and safe way to diagnose IgE-mediated allergies, with rare side-effects. Two cases of systemic allergic reactions during skin testing to food allergens are hereby reported. A 28-year-old male reported allergic reactions, mild to moderate in severity, each time he tasted fish in the frame of his professional duties. During SPT and prick-to-prick to raw and cooked fishes, he presented urticaria and tachycardia. A 59-year-old male had a long history of urticaria-angioedema and asthma attacks, following the consumption of mammalian meat. He was skin-tested to various meats and during the 5 last minutes of the test he developed generalized urticaria, allergic rhinitis and conjunctivitis. They were both advised to completely avoid the relative allergens. In conclusion, skin testing, particularly prick-to-prick, may cause anaphylaxis. Tests should be performed only by physicians with proper training in allergy, experienced in treating promptly and properly episodes of anaphylaxis.

Introduction

Skin testing is the diagnostic cornerstone that confirms or rules-out IgE-mediated allergy (1, 2). Skin prick tests (SPT) with commercial food-extracts are considered to be a safe, efficient and rapid method for screening purposes in IgE-mediated food allergy (2). The "prick-to-prick" (P-P) method is performed if extracts of specific food items are not available or differences in the allergenicity of different cultivar strains exist. In the P-P method, the tester pricks first the fresh food and then the skin (2). Intradermal skin tests with food are avoided because of increased risk of a systemic reaction; furthermote, intradermal food tests are characterized by increased sensitivity but low specificity (2, 3).

Although very rare, allergic reactions during skin testing have been reported and in most cases such reactions occur after P-P testing. Anaphylaxis during the performance of SPT to food extracts is extremely rare (4). Fortunately no fatalities during food allergy testing have been reported since 1984; the ones reported until then had occurred following intradermal tests (5).

In the following article two cases of allergic reactions during skin testing to food are reported. They were provoked by fish and meat P-P testing.

Cases description

Case 1

A 28-year-old male was referred for evaluation of adult onset fish allergy. Working as a cook, specialized in fish dishes, he reported repeated allergic reactions, mild (pruritus and later urticaria of the neck, axillary, genital areas) to moderate in severity (above skin symptoms + palpitations, tinnitus, gastrointestinal involvement); acute, self-limited episodes occurred each time he tasted fish or other seafood (in the frame of his professional duties), even though he did not swallow it and rinsed his mouth thoroughly. He claimed that he consumed canned tuna fish without problem. He is an atopic individual with a history of atopic dermatitis, seasonal rhinitis and asthma.

In vivo evaluation was undertaken with SPT to 4 commercial fish extracts (cod, salmon, trout and tuna, Stallergens, France) and P-P to the offending fishes (mackerel, bogue, salmon, anchovy, bassfish, brown picarel, comber, streaked gurnard and pandora, raw and cooked). Negative (50% glycerinated HAS-saline) and positive controls (histamine dihydrochloride, 10mg/mL) were used (in both cases). In vitro evaluation of specific IgE resulted positive to cod (18kU/L).

SPT were carried out on the upper back for adequate surface area with a sterile 1 mm-tip lancet (Stallergenes, France), followed by P-P tests, a quarter of an hour later. The SPT reactions were strongly positive to all commercial extracts (more than two times greater than the histamine's wheal and flare), with histamine's mean wheal diameter of 8mm. All fishes tested by P-P resulted positive with pseudopodia but were not outlined due to the reaction that followed.

Less than fifteen minutes after starting the P-P tests, he complained of pruritus and almost instantly he broke into giant urticaria involving the neck, axillary and genital areas; in addition mild tachycardia reproduced with accuracy the clinical picture he usually developed upon fish tasting; because of the rapid progression of the reaction, a single epinephrine dose (0.3mg) was administered SC and he remained heamodynamicaly stable. Cetirizine (10mg) and methylprednisolone (16mg) were also administered per os. He recovered uneventfully.

He was advised to move to another cooking area of the restaurant and completely avoid the contact and the ingestion of fish. A 'rescue set' containing a 5mg levocetirizine tablet, a methylprednizolone 16mg tablet and a self-injectable epinephrine, was prescribed to him.

Case 2

A 59-year-old male reported a 10 year history of generalized urticaria-angioedema and asthma attacks following the consumption of mammalian meats; they developed 2 hours after ingesting pork, beef, lamb, goat and rabbit. Since the onset of present illness, patient has tried twice to taste small amounts of beef and lamb, but 2 hours later he developed pruritus in the axillary area, angioedema, rhinoconjunctivitis and intense dyspnea. He tolerates dairies. He was referred to the allergist in order to reintroduce meat in his diet. Past medical history included: seborrheic dermatitis, gastric ulcer, coronary disease and symptoms of exercise-induced asthma.

SPT with 4 commercial extracts to milk, beef, pork, mutton (HAL Allergy, The Netherlands), as well as P-P with both raw and cooked meat (beef, pork, lamp and rabbit), were performed simultaneously on the volar surface of the forearm. SPT to milk was negative. All skin tests to mammalian meat resulted strongly positive, with a mean wheal diameter ranging 9-13mm, with pseudopodia (histamine= 7mm). During the last 5 minutes of skin testing, the patient developed facial pruritus and erythema, urticarial lesions of the trunk, itching red eyes, running nose and sneezing. Levocetirizine and methylprednisolone were administered per os and i.m., respectively and patient recovered in 15 minutes; he was instructed to strictly avoid the consumption of mammalian meat.

Discussion

The safety of skin testing is a common experience that has been confirmed by several studies. In a large survey of more than 18,000 subjects no adverse reactions due to skin testing to food extracts were reported (6). Results of another study, regarding 16,204 patients showed that vasovagal reactions may be noticed during skin testing in 0.04% of the patients (7). Allergic reactions during the performance of P-P are considered to be an extremely rare event, with an estimated prevalence of 0.008% in food allergic patients (8). A 6.5% rate of allergic reactions due to P-P testing has been reported among 92 babies, of less than 6 months of age, tested for food allergy (9). According to the findings of the same study, involving a total of 1,152 patients aged less than 19 years old, no reactions were reported in children older than 6 months. The authors concluded that infancy, the presence of atopic dermatitis and the performance of duplicate tests are risk factors for anaphylaxis during skin testing (9).

Mammalian meat allergy is an extremely rare food allergen, even among children allergic to cow's milk, which contains common proteins with meat (10). Thermo-labile proteins - like bovine serum albumin (BSA) and the bovine serum IgG - are considered the major allergens responsible for allergy to mammalian meat, in persons who report reactions after the ingestion of medium-rare or rare meat (10, 11). It appears that heat-resistant proteins are responsible for our Case's 2 allergy, since all episodes occurred upon consumption of well-cooked meat. Six of 24 protein fractions - with molecular weight 14-66kd - detected after SDS-PAGE of raw beef, were reported to be heat-stabile for up to 2 hours of heat (85° C) treatment (10).

Cross-reactivity in different mammalian meats has been reported with a frequency of 75.4% (12). Our Case 2 patient tolerates avian meat, but has reacted to different mammalian meats. That confirms the clinical cross-reactivity among mammalian meat proteins. The performance of multiple tests of cross-reactive food increased the local allergen load. Cautions could have been kept to prevent the reactions in both cases, like applying first wet pieces of food upon the skin without pricking them and perform tests gradually in more that one visits, first SPT followed by P-P (13); in Case 1 these cautions were not observed because of his clained tolerance to tuna fish).

In conclusion, facing the rare occurence of an allergic reaction during skin testing, physicians should avoid leaving the patient without surveillance, practicing tests without having the necessary emergency equipment and medication or testing infants with eczema and asthma using native food (2, 14). Tests should be performed only by physicians with proper training in allergy, experienced in treating promptly and properly episodes of anaphylaxis (14).

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