Genuine and cross-reactive allergenic molecules of birch and hazel

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Abstract:
Birch and hazel pollen are the most common causes of allergic diseases that occur during the spring season. For decades, the method of quantitative determination of specific IgE (sIgE) against allergen extracts has been used in the laboratory diagnosis of allergies. In recent years, there has been the possibility of determining sIgE by individual allergenic molecules, either native or recombinant. This method, known as *Component Resolved Diagnosis* (CRD), has made it possible to identify major and cross-reactive allergenic molecules.
Genuine allergenic molecules in birch (Bet v 1), hazel (Cor a 1) and hazelnut (Cor a 8, 9, 11, 14) are responsible for allergic reactions in most patients. Moreover, they cause a severe clinical picture in patients with hypersensitivity, in contrast to cross-reactive molecules, eg. Bet v 1 homologues in vegetables (celery, soy) and fruits (apple, cherry, pear), or Bet v 2 homologues in vegetables (celery, carrot) and fruits (muskmelon, peach, banana), that cause milder symptoms.
In the diagnostic context, future research will make it possible to determine the diagnostic significance of the detection of individual allergen molecules responsible for allergy symptoms in each individual patient.
The application of bioinformatics will certainly help in the interpretation of the findings, especially in cases when multiplex assays are applied. Particular progress is also expected in the application of allergen molecules to allergen-specific immunotherapy.

Keywords: allergy; birch pollen; hazel pollen; hazelnut, component resolved diagnosis

Sažetak:
Prave i križno-reaktivne alergenske molekule breze i lijeske
Pelud breze i lijeske najčešći su uzročnici alergijskih bolesti koje se pojavljuju tijekom proljeća. U laboratorijskoj dijagnostici alergija već se desetljećima koristi metoda kvantitativnog određivanja specifičnog IgE (sIgE) prema alergenskim ekstraktima. Posljednjih godina postoji mogućnost određivanja sIgE prema pojedinačnim alergenskim molekulama, bilo nativnim ili rekombinantnim. Ova metoda, poznata kao komponentna dijagnostika (CRD), omogućila je identifikaciju glavnih i križno-reaktivnih alergenskih molekula.
Prave alergenske molekule breze (Bet v 1), llijeske (Cor a 1) i lješnjaku (Cor a 8, 9, 11, 14) odgovorne su za alergijske reakcije kod većine pacijenata. Štoviše, kod pacijenata s preosjetljivošću izazivaju tešku kliničku sliku, za razliku od križno-reaktivnih molekula, npr. homologa Bet v 1 iz povrća (celer, soja) i voća (jabuka, trešnja, kruška) odnosno homologa Bet v 2 iz povrća (celer, mrkva) i voća (dinja, breskva, banana), koji uzrokuju blaže simptome.

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U dijagnostičkom kontekstu, buduća će istraživanja omogućiti utvrđivanje dijagnostičke značajnosti otkrivanja pojedinačnih alergenskih molekula odgovornih za simptome alergije kod svakog pojedinog pacijenta.
Primjena bioinformatike zasigurno će pomoći u tumačenju nalaza, osobito u slučajevima kada se primjenjuju multipleks-testovi. Poseban napredak očekuje se i u primjeni alergenskih molekula u alergen-specifičnoj imunoterapiji.

Ključne riječi: alergija; pelud breze; pelud lijeske; lješnjak; komponentna dijagnostika

Introduction

Pollens play a major role in the onset of seasonal allergic disorders, mediated by IgE (eg. conjunctivitis, rhinitis and/or asthma) all over the world (1,2). The most clinically relevant allergenic plants in Europe are grasses, trees and weeds (3).
Springtime allergies are caused by tree pollen that mostly belong to the orders Fagales (birch, hazelnut, alder, beech, oak), Proteales (plane tree, sycamore), Pinales (cypress), and Lamiales (ash, olive, lilac) (4). The order Fagales encompasses two main families - Betulaceae and Fagaceae; Betulaceae includes the genera Betula (birch), Corylus (hazel), Alnus (alder), Carpinus (hornbeam), and Ostrya (hop-hornbeam). The family Fagaceae comprise the genera Quercus (oak), Castanea (chestnut), and Fagus (beech) (5,6). Among order Fagales, birch, alder and hazel are the most potent cause of tree pollen allergy. During pollination, the onset of symptoms in hypersensitive patients depends on the number of allergenic grains in the air. The birch pollen concentrations greater than 30 grains/m^3/24 h trigger severe symptoms, and values greater than 80 grains/m^3/24 h produce allergy symptoms in 90% of patients. The hazel pollen causes allergic reaction at concentrations between 20 and 30 grains/m^3/24 h (7). The threshold value for the occurrence of allergy symptoms in subjects with hypersensitivity to alder pollen is 45 pollen grains in m^3/24 h) (8).
In Croatia, birch pollination was found to be most pronounced in the period from February to May, with peak grain count in April (7). The highest pollination of alder and hazel was recorded during February and March. About 70% of patients with rhinitis / asthma caused by hypersensitivity to birch pollen have an associated hypersensitivity to cross-reacting food allergens (9).
The basic laboratory diagnosis of allergies is based on 1. the detection of the type of allergic reaction (IgE-mediated allergies or non-IgE-mediated allergies) and 2. the detection of the triggers that led to allergic reaction (determination of IgE against allergen extracts and allergenic components) (10). This is followed by 3. the detection of cross-reactivity between individual allergens or allergenic components or molecules, respectively, Figure 1 (11).

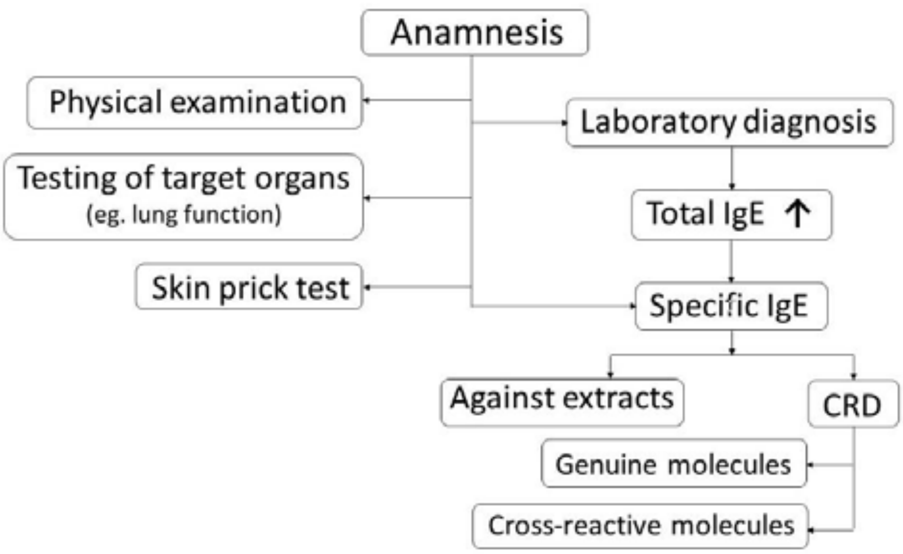

Figure 1. Simplified allergy diagnosis algorithm. In laboratory diagnostics, only procedures related to IgE-mediated allergies are shown; CRD – component resolved diagnosis.

Persons with atopy have an increased concentration of total IgE (tIgE) and specific IgE (sIgE) against causative allergens. When determining the concentration of either tIgE or sIgE, it is important to keep in mind the time elapsed since exposure to the causative allergen. (12,13). Everyday practice has shown that IgE concentration wass always significantly higher 2 to 6 months after exposure to seasonal allergens. After that time, its concentration begins to decrease.

Component Resolved Diagnosis, CRD, or Molecular Allergy Diagnosis, MAD, respectively, for determination of serum sIgE concentration against purified natural and recombinant allergenic molecules is used recently (14 - 16). Molecules of natural (n) allergens are obtained by purification of allergen extracts using chemical, chromatographic, electrophoretic and / or immunoaffinity methods. The production of recombinant (r) allergen molecules is a complex process that includes 1. extraction and isolation of messenger RNA (mRNA) from allergen sources, 2. synthesis of complementary DNA (cDNA), 3. electrophoretic separation of individual components of allergen sources, 4. preparation of primers for polymerase chain reaction (PCR), 5. amplification of cDNA of individual allergenic components, and 6. expression of (r) allergens (eg. rBet v1, rBet v2, rBet v4, etc.). CRD thus allows detection of sIgE to potential causative allergenic molecules that trigger an allergic reaction in a person with atopic disese.

Allergen Nomenklature

Allergen name consists of three letters from the genus, one letter from the species epithet and an Arabic numeral, eg. Bet v 1= *Betula verucosa* (the major allergen from birch pollen), Cor a = *Corylus avellana* (the major allergen from hazel pollen). The number is given in order of their discovery, so these mentioned allergens were the first discovered allergens from birch and hazel pollen, respectively (17,18).

Allergen Definiton

Allergens are proteins, glycoproteins, lipoproteins or conjugates of proteins and haptens, with a known nucleotide and / or amino acid sequence, carbohydrate composition and relative molecular mass (Mr 5 - 150 kDa) (16,19).

According to the chemical structure, major allergens can be classified into a list of different structural families. Some of them are, for example: pathogenesis-related class 10 proteins (PR-10-P), protease inhibitor/seed storage/lipid transfer proteins (LTP), cupin superfamily (11S and 7S plant seed storage globulins), lipocalin/cytosolic fatty-acid binding protein family, EF hand family, papain-like cysteine protease, profilins, thaumatin-like proteins, group 1/2/3 grass pollen allergen i.e. expansin family, expansin with N-terminal domain, group 5/6 grass pollen allergen, glycosyl hydrolases family 17 (endo-beta-1,3-glucosidase), hyaluronidase, cyclophilin type peptidyl-prolyl cis–trans isomerase, and FAD linked oxidase/berberine bridge enzyme-like family, ect. (19).

In clinical practice, it is important to distinguish genuine (primary) sensitization and cross reactivity, particularly in presumed polysensitizations. Today, the allergist has the possibility to refer a patient who has symptoms during the spring season to a laboratory diagnosis i.e. determination of sIgE against genuine allergen molecules and cross-reactive molecules of birch and hazel pollen, but also allergen molecules present in plant-food, especially in patients who have plant-food syndrome. Table 1. shows genuine and cross-reactive allergenic molecules from different allergen families with associated data on the stability of individual molecules according to heat and digestion, as well as the risks and severity of symptoms that can trigger in patients with hypersensitivity.

Table 1. Major allergen families which include allergens of birch, hazel and hazelnut

Allergen family	Genuine molecules	Cross reactive molecules
Profilins	Birch pollen (Bet v 2) Bet v 2 homologues: *Tree pollen*: olive (Ole e 2, goosefoot (Che a 2)	Bet v 2 homologues: *Vegetables*:* celery (Api g 4), carrot (Dau c 4) *Fruits*:* muskmelon (Cuc m 2), peach (Pru p 4), banana (Mus xp 1) *Seeds*:* mustard (Sin a 1)
PR-10-P	Birch pollen (Bet v 1) # Bet v 1 homologues: *Tree pollens*; alder (Aln g 1), hazel (Cor a 1), hornbeam (Car b 1), chestnut (Cas s 1), beech (Fag s 1), oak (Que a1) cypress (Cup a 1),	Bet v 1 homologues: *Fruit* (apple (Mal d 1), cherry (Pru av 1),pear (Pyr c 1), stone fruits, eg. peanut (Ara h 8); V*egetables*:* celery (Api g 1), soy (Gly m 4)
nsLTPs	Stone fruit: Hazelnut (Cor a 8)	Stone fruit Hazelnut (Cor a 8) cross-reactive with peach (Pru p 3), both ##
Storage proteins	Stone fruit Hazelnut (Cor a 9, Cor a 11, Cor a 14) ##	

– Marker of genuine (species-specific) sensitization; ## – risk- or severity-associated molecules; * – sensitive to heat/digestion

According to the chemical structure, allergenic plant molecules are classified into several families, eg. profilins, pathogenesis-related proteins (PR-10-P), non-specific lipid transfer proteins (nsLTPs), storage globulins (eg. 7S seed storage globulin, 11S seed storage globulin), binding proteins (eg. polcalcins) (Table 2.). Some plant allergens are enzymes, eg. phenyl coumaran benzylic ether reductase, cyclophilin (peptidyl-prolyl isomerase), glutathione-S-transferase (Table 3.). Allergens are characterized by acidic isoelectric point, pI = 5.7±0.15 (with exception nsLTPs) and negative electrostatic potential (20). These molecules are responsible, for example, for plant growth, diferentiation, defense of the plant from bacteria, viruses and fungi.

Profilins are smal actin-binding proteins (Mr 14-17 kDa) present in all eukaryotic cells. Profilin isoforms comprise 100 - 131 amino acids. They play an important role in normal cell growth, differentiation, proliferation, motility, and cytokinesis (21 Krishnan). Those present in plants have a homology higher than 75%. Only 10-20% of persons sensitized to pollen have IgE against profilins. In these subjects, the cross-reactivity between pollen, vegetables and latex is very high. They cause mild symptoms after inhalation, contact or ingestion. Severe symptoms have not been described (6).

PR-10 proteins are small (Mr about 17 kDa; contain154-163 amino acids), slightly acidic proteins resistant to proteases (22). These proteins are widely distributed in the plant world and represent defensive proteins, and are found in the reproductive tissues of the plant (pollen, seeds and fruits). PR-10 protein molecules are thermolabile. On the surface of some homologous molecules there are epitopes which, after inhalation or ingestion, mediate an allergic reaction via IgE (6).

nsLTPs are classified in two subgroups by Mr, i.e. LTP1s (Mr 9-10 kDa; contains 90-95 aminoacid residues) and LTP2s (Mr 7 kDa; contains 70 amino acids). Both subgroups are present in plants. In addition to having the ability to transfer membrane lipids, they are responsible for defending plants from bacteria, fungi and viruses (23). They are resistant to digestion by intestinal enzymes and to temperature.

Polcalcins belong to the family of calcium-binding proteins. They can have two, three or four calcium-binding domains, and the monomer has a molecular weight of 8 to 9 kDa, (24). Allergens from the polcalcin family are highly cross-reactive, which is especially pronounced in pollen tissues, but not in plant-derived foods.

Genuine and Cross-Reactive Allergen Molecules

The allergenicity of an allergenic molecule can be defined as the potential of that molecule to trigger an allergic reaction. Allergenicity depends on the structure of the allergenic molecule, i.e. size (Mr), acidity (pI), charge, solubility, 3D protein-folding, and stability (20,25).

Until the introduction CRD (MAD) of allergies, allergens were classified as major and minor allergens, taking into account two criteria: the IgE-binding frequency and the induction of immediate skin prick test response. According to the first criterion, "major" allergens can sensitize more than half of individuals with atopy (> 50% IgE-binding), in contrast to "minor" allergens that can sensitize less than half of allergic persons (<50% IgE-binding), and according to another criterion, "major allergens" can induced immediate skin test responses in > 90% of allergic individuals, and "minor allergens" in <20% of patients (26). Certainly, the major epitope of allergenic molecule should be located at the molecular surface, so that it could be accessible to its sIgE.

CRD has advanced the accurately define the genuine sensitization to allergens, and has made it possible to distinguish between genuine (primary or marker molecules) and cross-reactive allergenic molecules. Genuine pollen allergen molecules are those allergenic molecules that are responsible for family- or species-specific sensitization (6). They induce sensitization and the production of sIgE, and triggers a reaction between the allergenic molecule and its sIgE, which ultimately leads to the appearance of allergy symptoms.

Cross-reactive allergenic molecules are similar (homologues) to genuine molecules, belong to the same genus, and can trigger an allergic reaction when the similarity with the genuine molecule is greater than 70%. If their similarity is less than 50% cros-reactive molecules will not trigger an allergic reaction (25). Cross-reactive allergenic molecules can cause an allergic reaction after previous contact of patient with the main sensitizer (6). Cross-reactive molecules are found in plant fruits, and are similar to the genuine molecule in pollen. Because of their similarity, the IgE molecule recognizes both, the genuine allergen molecule in pollen and a similar molecules in vegetables, fruits and nuts. If, for example, a patient is allergic to birch pollen molecule, he may develop an allergic reaction after consuming the fruit, eg. apple. Allergic reaction to cross-reactive allergenic molecules may be less severe in comparison with the reaction to genuine allergenic molecule. Cross-reactivity can also be triggered by cross-reactive carbohydrate determinants (CCDs), but this reaction is not clinicaly relevant (27).

Reaction between sIgE and cross-reactive allergenic molecule is of lower affinity than reaction between sIgE and genuine allergenic molecule. The cross-reaction between evolutionarily unrelated pollen allergens and plant foods is caused by allergens collectively called panallergens. The reaction occurs due to the homologous structure of molecules from these different sources. Phylogenetically, these are very old, conservative proteins. According to current knowledge, the studied panallergens include a few protein families, eg. profilins, PR-10 proteins, nsLTP and polcalcins.They exhibit IgE-mediated cross-reactivity between evolutionarily unrelated species distributed in the plant world, eg. unrelated pollen and plant food allergen sources (24). Their main role is manifested in very important defensive or metabolic functions (Table 2.).

Table 2. Basic characteristics of plant allergenic proteins

	Profilins	PR-10 proteins#	nsLTPs#	Polcalcins
Allergen source	Timothy grass pollen	Birch pollen	pomegranate; Peach: seeds, fruits, leaves, roots, pollens	Pollens of trees, grasses and weeds
Mr (kDa)	14 - 17	17	LTP1s: 9-10; LTP2s: 7	8 - 9
Length (amino acids)	100 - 131	154-163	LTP1s: 90-95; LTP2s: 70	78 (monomer)
Isoelectric point	5.07	5.39	9.25 (8.8 to 12)	4.39
3D structure	No	Yes	Yes	Yes
Molecular structure	Central 6-stranded β sheet and two α-helices	Seven-stranded anti-parallel β-sheet flanked by three α-helices	C-terminal alpha-helices, 6 - 8 cysteine residues	α-helices
Disulfide bonds	Yes	No	Yes	No
Glycosylation	No	No	No	No
Heat and digestion resistant	Thermally labile	Thermolabile/ resistant to proteases	High resistance to gastrointestinal proteolysis, to pH and high temperatures	Stable to thermal denaturation
Function	Cell growth, differentiation, proliferation, motility, and cytokinesis	Protective role against pathogens bacteria, fungi and viruses	Transfer membrane lipids; defending plants from bacteria, fungi and viruses	Control of intracellular calcium levels during pollen germination
Cross-reactivity	Between different pollen sources and some plant foods, and latex	Some pollens (within order Fagales), plant food (mostly fruits and some vegetables)	Within nsLTP family; in plant food and vegetables, but also in pollen and latex	Within pollens, only
Allergy symptoms	Mostly mild (rhinitis, conjunctivitis)	Mild to severe (conjunctivitis, rhynitis, asthma)	High risk of severe systemic reactions (angioedema, anaphylaxis), following the intake of foods (raw, cooked or preserved foods)	Mild to severe respiratory symptoms; marker of polysensitisation; no connection with food allergy

– major allergens; 3D – three dimensional structure available.

Allergenic Birch Pollen Molecules

Currently, seven allergen molecules have been defined in birch pollen (18,28 - 33). They belong to different protein families, i.e. PR-10-P, profilins, polcalcin-like proteins, phenyl coumaran benzylic ether reductase, cyclophilin and glutathione-S-transferase, as shown in Table 3. Among them, Bet v 1 belongs to genuine allergenic molecules, and other molecules are cross-reactive. Bet v 1 homologous molecules can be found in pollen of related *Fagales* trees (hazel - Cor a 1, alder Aln g 1, beech - Fag s 1, oak - Que a 1, hornbeam - Car b 1, chestnut - Cas s 1).

In addition, it should be mentioned that allergenic molecules homologous to the Bet v 1 molecule can be found in fruits and vegetables, such as *Rosaceae* fruits (e.g. apple - Mal d 1, pear - Pyr c 1, cherry - Pru av 1, apricot - Pru ar 1), *Apiaceae* vegetables (e.g. celery- Api g 1, carrot - Dau c 1), as well as in nuts, seeds, legumes (eg. hazelnut - Cor a 1, peanut - Ara h 8, soybean - Gly m 4 (6,24). Amino acid identities between Bet v1 and its homologous molecules from the PR-10-P family (Bet v 1, Mal d 1, Pru av 1, Act d 8. Api g 1, Ara h 8, Gly m 4 and Cor a 1) are between 39 and 67%.

Table 3. Genuine and cross-reactive allergenic molecules in birch pollen (adapted according to the references 18,28-33.

Allergenic molecule	**Protein**	**Mr (kDa)**	**Allergenicity / cross-reactivity** ** Prevalence among birch pollen allergic patients
Bet v 1	PR-10-P (Bet v 1 family member)	17	95 - 100 % of patients have sIgE to Bet v 1
Bet v 2	Profilin	15	22% of patients have sIgE to rBet v 2. Bet v 2 induces histamine release from blood basophils of profilin-allergic individuals, but not of individuals sensitized to other plant allergens.
Bet v 3	Polcalcin-like protein	24	Approximately 10% of patients show IgE binding to rBet v 3
Bet v 4	Polcalcin	7-8	5% of patients have sIgE to rBet v 4
Bet v 6 #	Phenyl coumaran benzylic ether reductase	33	32% of patients have sIge ≥3.5 kUa/L (≥ class 3) to rBet v 6
Bet v 7	Cyclophilin (peptidyl-prolyl isomerase)	18	20.8% of subjects recognize Bet v 7. Patients demonstrate positive SPT with birch pollen extract.
Bet v 8	Glutathione-S-transferase	27	13% birch pollen allergic subjects recognise rBet v 8

S IgE – specific IgE; # – previously known as Bet v 5; ** – tested in vitro; r – recombinant molecule.

Allergenic Hazel Pollen and Hazelnut Molecules

According to the Allergen Nomenclature Sub-Comittee three alergenic molecule have been defined in hazel pollen and eight in hazelnut (18). They all belong to different protein families, i.e. PR-10-P (Cor a 1), isoflavone reductase homologue (Cor a 6), ns-LTPs (Cor a 10), profilins (Cor a 2), luminal binding protein (Cor a 8), seed storage globulins (Cor a 9, Cor a 11), oleosins (Cor a 12, Cor a 13, Cor a 15), albumins (Cor a 14) (Table 4.). Hazel pollen allergenic molecule Cor a 1 belongs to major and genuine allergenic molecule, respectively. It should be noted that Cor a 1 belongs to the group Bet v 1- homologous allergenic molecules. Amino acid identities between Cor a1 and its homologues from the PR-10-P family (Bet v 1, Mal d 1, Pru av 1, Act d 8. Api g 1, Ara h 8 and Gly m 4) are between 43 and 67%. (EAACI). Among plant fruit allergens, Cor a 9 is also a major allergen. Genuine allergenic molecule Cor a 1 has two isoforms, with a closely related sequence identity; one is found in hazel pollen, and the other in hazelnut (6,34 - 36). Cor a 1, is heat labile, and also unstable to gastric digestion.

Table 4. Genuine and cross-reactive allergenic molecules in hazel and hazelnut allergens (adapted according to the references 6,18,34 - 36.

Allergenic molecule	Protein	Mr (kDa)	Allergenicity / cross-reactivity **
	Pollen molecules		
Cor a1	PR-10-P (Bet v 1 family member)	17	100% patients allergic to hazelnut show IgE binding to rCor a 1
Cor a 6	Isoflavone reductase homologue	35	No data available
Cor a 10	nsLTP type 1	70	Between proteins from different pollens and plant foods
	Food molecules		
Cor a 2	Profilin	14	15.4% off hazelnut allergic patients had IgE biding to rCor a 2
Cor a 8	nsLTP	9	Some patients with severe anaphylactic reactions to hazelnut showed sIgE binding to Cor a 8
Cor a 9	11S seed storage globulin (legumin)	40	86% off hazelnut allergic patients with systemic reactions had IgE biding to rCor a 9
Cor a 11	7S seed storage globulin (vicilin)	48	Cross-reactivity with Cor a 1 in about half of patients
Cor a 12	17 kDa oleosin	17	63% of patients with hazelnut and/or peanut ingestion related symptoms.
Cor a 13	14-16 kDa oleosin	14-16	63% of patients with hazelnut and/or peanut ingestion related symptoms.
Cor a 14	2S albumin	10	1/3 of hazelnut allergic individuals had IgE that reacted with natural 2S albumin from hazelnut.
Cor a 15	Oleosin	17	Found in all hazelnut SPT positive children

Cor a – *Corylus avellana*; PR-10-P – Pathogenesis-related protein; nsLTP – non-specific lipid transport protein; ** – tested in vitro; SPT – skin prick test.

Identification of Allergenic Molecules

Identification of partcular genuine and cross-reactive allergenic molecules became possible after individual allergen molecules were isolated from allergen sources and after production of some recombinant allergenic molecules. Subsequently, methods have been developed for the determination of sIgE precisely according to these particular molecules (i.e. CDR). CRD is based on a sandwich fluoro-immunochemical or lumino-immunochemical method on a three-dimensional carrier. It can be performed as a singleplex (one sample - one allergenic molecule) and multiplex assay. The latter assay involves the simultaneous determination of the concentration of sIgE against a great number (up to 120) of allergenic molecules (37).

Recently, CRD has been increasingly used in laboratory diagnosis of allergies. The choice of allergen for singleplex assay is made based on the anamnesis, clinical findings of a patient and on the results of a skin prick test (SPT). The results are expressed in semi-quantitative ISAC Standardized Units for sIgE (ISU-E), based on the World Health Organization IgE standard (WHO 75/502). The detection range is 0 - 100 ISU-E. In the future, computer programs could help in interpretation of multiplex assays results, which would significantly help physicians in their daily practice.

Allergenic Molecules in Clinical Practice

Indirect detection of allergenic molecules by determining the concentration of sIgE has brought significant advances in understanding the immune mechanisms involved in allergic diseases. It is important for clinical practice to know whether a patient is hypersensitive to genuine allergenic molecules, cross-reactive molecules. In addition, it should be determined whether the patient has simultaneous hypersensitivity to allergenic molecules from different allergenic sources, eg. weeds and birch, i.e. co-sensitization (38,39). It is now possible to detect hypersensitivity to genuine or cross-reactive allergenic components, which is not possible either with SPT or with the determination of sIgEs against allergenic extracts. CRD allows physicians to more accurately interpret severe, moderate, or mild allergy symptoms in each individual patient, complete a previous diagnosis based on allergen extract diagnostics (using both SPT and sIgE), and adjust individual symptom prevention (advising the patient to avoid contact with causative allergens) and treatment of diseases, including allergen specific immunotherapy (ASIT).

Identification of genuine and cross-reactive molecules may be useful in subjects with: i) anaphylaxis caused by non-steroidal anti-inflammatory drugs, effort, or with idiopathic anaphylaxis; ii) delayed anaphylaxis (eg. 3 - 6h after consummation of red meat); iii) multiple hypersensitivity, i.e. sensitivity to pollen and plant food allergens; iv) latex allergy; v) food allergy (40,41). In addition, CRD should be applied when selecting allergens for ASIT.

The severity of the symptoms depends on the family to which the causative allergen molecules belong. It is known that storage proteins (genuine allergens) are generally responsible for the severe and long-lasting symptoms. Marker of genuine (species-specific) sensitization is Bet v 1. Milder and shorter-lasting symptoms are caused by cross-reactive allergens, The severity of symptoms in hypersensitivity to the allergenic molecules of birch and hazel increases from profilins to storage proteins, i.e. profilins < PR-10-P < nsLTPs < storage proteins) (Figure 2.) (6,36).

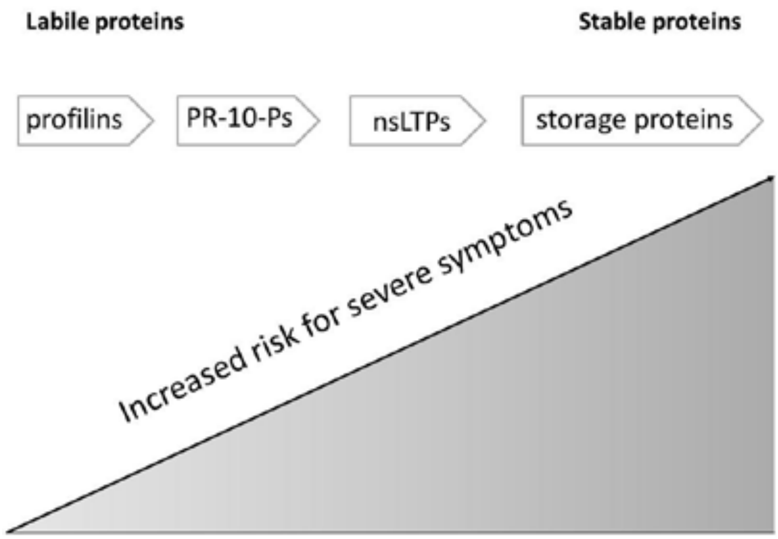

Figure 2. Relationship between the severity of symptoms and the family to which individual allergen molecules belong. PR-10-Ps - Pathogenesis-related proteins; (Adapted according to the ref.)

Risk- or severity associated allergenic molecles families are 2S albumins (Cor a 14 (hazelnut), Gly m 8 (soy), other seed storage proteins (Cor a 9, Cor a 11, Gly m 5, Gly m 6), nsLTP (eg. Pru p 3, peach), Cor a 8 (hazelnut).

Pollen-food allergy syndrome occurs in patients with panallergen allergy, especially in hypersensitivity to allergen molecules from the profilins, PR-10s, and nsLTPs families (42). It should be noted that stone fruits, seeds and legumes contain stable seed storage proteins (2S albumins, 7S and 11 S globulins), which are involved in direct sensitization of subjects with atopy, and can therefore cause more severe symptoms. Although hazelnut allergenic molecules Cor a 14 and Cor a 9 belong to minor allergens, they can cause clinically significant allergy symptoms due to their chemical structure (2S albumin and 11 S globulin, respecitvely) (6).

Moderate symptoms are manifested on the conjunctiva (itch, tearing, redness), nose (itch, sneezing, runny nose, stuffy nose), ears, eg. internally, Eustachian tubes (itch), eye lids, lips, cheeks, earlobe, face (tissue swelling / angioedema). Extremely rarely, an allergic reaction can be systemic, which means the appearance of symptoms such as localized, multifocal or generalized angioedema, nausea, vomiting, diarrhea, abdominal pain, breathing difficulties, wheezing, cough, general weakness. Mild symtoms are itch of lips mucosa, oral mucosa, palate, burning, stinging of palate and throat or mild mucosal swelling at all mentioned localizations.

nsLTPs can cause allergy symptoms of varying severity, from mild pollen food allergy syndrome (PFAS) to anaphylaxis (36,43). Due to cross-reactivity between homologous allergenic molecules, patients hypersensitive to tree pollen (birch, hazel and alder) may experience transient oropharyngeal symptoms (mostly PFAS) after consumption of particular Bet v 1-related plant fruits (eg apple, pear, apricot, cherry, plum, hazelnut, walnut, almond, mango, avocado, kiwi) and vegetables (tomatoe, carrot, fennel, celery, soybean), those containing PR-10-P. Generally, symptoms appear often within a few minutes, (sometimes immediately) after consumption of raw plant food, not after being heat-treated or acidified (6). Symptoms of PFAS can be mild and transient, moderate and severe.

Allergenic molecules similar to Cor a 1 might be also expressed in hazelnuts, so that PFAS caused by hazelnut allergenic molecules may be also present in patients with hypersensitivity to hazel pollen. These patients frequently suffer from mild PFAS (44). Except of homology between Cor a 1 and Bet v 1, it was found that there is also homology between Cor a 2 (profilin) and Bet v 2 (45). Three hazelnut allergens responsible for severe symptoms have been recently described. These are Cor a 8, (nsLTP), Cor a 9 (11 S globulin-like seed storage protein) (44) and Cor a 14 (46 Faber MA).

Currently, five allergens (Cor a 1, Cor a 8, Cor a 9, Cor a 11, and Cor a 14) from hazel/hazelnut considered to be the major culprits associated with reactions in patients sensitized to hazelnut (34) Recently, it was showed that Cor a 14 (2 S albumin) was superior to Cor a 9 in predicting moderate – to severe reaction in hazelnut allergic patients. In patients with history of birch pollen allergy and/or sensitization to birch pollen, hazelnut sensitization is frequent due to cross-reactivity (PR-10 proteins) and by negative IgE testing for Cor a 9 and Cor a 14, true hazelnut allergy can be ruled out. (47). Coallergy to peanuts, three nuts, and/or sesame seeds may be due to immunologic cross-reactivity between 2 S albumins, and physicochemical and structural similarities of the 2 S albumins from different foods support the thesis that coexistence of these allergies is due to structural basis (48). In areas lacking exposure to birch pollen (e.g. Western Mediterranean areas) cross reactivity between the LTPs of the peach Pru p 3 and hazelnut Cor a 8 is mainly reason for the severe reactions to hazelnut (49)

Interestingly, the sensitization to Cor a 11 in a birch-endemic region is age-dependent and predominantly it occurs in children with severe hazelnut allergy and in preschool children with PFAS, but is absent in adults with PFAS related to their underlying birch pollen allergy (50)

The clinical picture of the patient is also crucial for the final decision on management and treatment. In case of PFAS, treatment opinions include food avoidance, education, antihistamines, epinephrine auto injector (EPI), and ASIT. In patients with isolated oropharyngeal symptoms, testing for sensitization and prophylactic avoidance of potentially cross-reactive foods is generally not recommended. Patients are instructed to avoid the specific raw fruits or vegetables or the nuts (raw and thermally processed) that have caused symptoms in the past (51,52) Patients who have previously eaten thermally processed (cooked, microwaved, pasteurized, or baked) fruits and vegetables without side effects may continue to eat these forms of the food (53). According to the literature, in patients with PFAS risk for systemic reactions is estimated to be between 2 and 10 percent. In case of patient with PFAS who have experienced systemic reaction, it is suggested a strict avoidance of the offending foods in all formula (42) Tolerance to cross-reactive foods should be carefully evaluated and clear instruction given of what to avoid. EPI is recommended in the following situations i) in patients with past systemic reaction, ii) allergy to peant, tree nuts and mustard, iii) reactions to cooked plant food and iv) sensitization to peach and apple in Western Mediterranean areas.

Prevention of allergy symptoms implies avoiding the consumption only those Bet v 1 homologues of raw plant foods, which have caused allergic signs and symptoms.

Allergen specific immunotherapy is the only etiological therapy of allergic diseases with long-term and post-treatment benefits. Several trials have demostrated that subcutaneous *immunotherapy* (SCIT) or *sublingual Immunotherapy* (SLIT) with birch alone or bitch, alder and hazel mixture is effective treatment for respiratory allergy. One trial with recombinat Bet v 1 have demostrated similar clinical efficacy supporting the thesis that major allergen is the main cause of disease. Recently, it is suggested that ASIT with birch pollen extract is also effective treatment of Fagales tree pollen allergy due to high degree of IgE cross-reactivity of the tree species (54).

However it is not clear that ASIT with tree pollen extracts has a beneficial effect on cross-reacting plant food allergies. (55). Therefore, PFAS caused by Bet v 1 homologous allergenic molecules from plant foods without the presence of pollen induced respiratory symptoms is not an indication for ASIT in patients with tree pollen allergy (56,57).

Although, there are some evidence that sublingual ASIT to pollen allergens has some positive effects in terms of T-cell tolerance, immune deviation, and regulatory T cells, as well as allergen-specific IgG4 further prospective studies will be needed to obtain definitive confirmation (42).

Conclusion

Identification of genuine and cross-reactive allergens makes it possible to predict or assess the risk for the severity of allergy symptoms and their duration. Today it is known that milder and shorter symptoms are initiated by cross-reactive allergens, and that severe and long symptoms are initiated by genuine allergenic molecules. The severity of symptoms in hypersensitivity to the allergenic molecules of birch and hazel increases from profilins to storage proteins, i.e. profilins < PR-10-P < nsLTPs < storage proteins). Storage proteins are generally responsible for the severe symptoms. It is not yet clear that ASIT with pollen allergen extracts has a beneficial effect on cross-reacting plant food allergies.

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