CHARACTERISATION OF POLLEN ALLERGENS

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Abstract: Allergy is hypersensitive reaction by the body to foreign substances (antigens) which in similar amounts and circumstances are harmless within the bodies of other people. The allergic response develops when the natural immune defence mechanism, responsible for the correct reaction to environmental agents, is disturbed. The allergens are divided into those originating from the natural environment and those from a chemically contaminated environment. The most frequent allergens from the natural environment are inhalant ones present in pollen grains, mould fungi spores and in fragments of mycelial hyphae. The airborne allergens also include: bacteria, house dust mites, epidermis of house pets, allergens of some food products and insect venom. The allergens originating from the natural environment are usually proteins, being high-molecular compounds of molecular weight higher than 10 kDa. Pollen allergens are water-soluble proteins or glycoproteins of molecular masses from 10–70 kDa. Many of them are resistant to pH changes and high temperature, even up to 100°C. Apart from pollen grains, allergens can occur in other parts of plants: roots, stems, leaves, seeds or fruit, in substances excreted by plants, such as juice and volatile oils, or in other bioaerosols of plant origin, e.g. fluids released during treatment of some crops. Proteins of some antigens show some analogies in the amino acids sequence, which determine immunological similarity and cross reactivity. From among factors conducing pollen allergy the most important are genetic and environmental ones (air pollution, exposure to allergens, infections of respiratory tract, diet) and microflora of pollen grains.

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INTRODUCTION

Development of civilisation, often at the expense of the natural environment by pollution, stimulates the appearance of new health problems, among others an increase in cases of allergy diseases. The number of people suffering from allergy reaches 15–30% of population and pollinosis - the most often observed - occurs in about 10–15% of the inhabitants of our planet [22, 52]. Irrespective of the type of symptoms, allergies are chronic diseases weakening the physical condition and the ability to concentrate in the sufferers. Fighting with them may demand a change of lifestyle, or even profession, adhering to a diet and maintaining allergen avoidance, long-term symptomatic treatment and immunotherapy [13, 29, 51, 52, 95].

Minimisation of the symptoms of pollen allergy is strictly related to avoidance of exposure to large doses of the allergen. The knowledge of the potentially allergenic pollen count and its changes throughout the pollination period in a given area is of great importance for allergic persons, and for determination of the origins of the disease and recommendation of an effective therapy [4, 52, 58]. The recognition of allergens’ properties, mechanisms of pollen allergy and factors conducing their appearance is of great importance in prophylaxis of allergies (allergic diseases) becoming a social problem on all continents [13, 35, 62].
MECHANISM OF POLLINOsis DEVELOPMENT

Allergy is hypersensitive reaction to foreign substances (antigens) which in similar amounts and circumstances are harmless within the bodies of other people. The term allergy was proposed in 1906 by Clemens von Pirquet to describe the state of altered reactivity of an organism. It combines two Greek words: allos - other and ergos - work, action [52].

The allergic response develops when the natural immune defence mechanism, responsible for the correct reaction to environmental agents, is disturbed. The natural immunity of the human organism is a result of the congenital immunity and the immunity acquired in contacts with environmental antibodies. The immunity has two components:

- The humoral - due to antibodies, especially those belonging to the immunoglobulin classes IgG, IgA and IgM, and to a complement system.
- The cellular immunity - depending on the cells involved in the immunity reactions such as presenting antigen cells (macrophages, dendritic cells), lymphocytes T and B, monocytes and granulocytes [33].

Apart from the humoral and cellular immunities, the human organism has specialised mechanisms of local immunity in the organs in direct contact with the environment, e.g. local immunities of mucous membranes of the respiratory tract, alimentary tract and skin. In healthy people there is a physiological balance between the amount and the functional state of the cell mediated immune system. In allergic reaction this statement is disturbed. According to the classification proposed by Gell and Coombs [24], there are four types of allergic reactions:

**Type 1** (immediate, anaphylactic), most often observed in pollinosis; develops when the allergen stimulates the organism to produce specific antibodies belonging to immunoglobulins class E (IgE) [33, 40, 52, 67]. These specific IgE antibodies bind specific allergen on the surface of mastocytes and basophiles, and stimulate them to release inflammatory mediators (early phase of allergic reaction). When the organism is still exposed to the allergen, the late phase of type 1 allergic reaction develops followed by chronic inflammation. In this phase, excretion of inflammatory mediators can be induced not only by the specific allergen that has triggered the reaction, but also by unspecific stimuli, e.g. chemicals (pesticides, formaldehyde, phenol) or physical factors (effort, decreased temperature, compression).

Clinical symptoms observed in type 1 allergic reaction are: nose itching, sneezing, burning sensation, production of watery or mucus excretion, conjunctivitis, and possibly altered condition of other organs [6, 95]. Type 1 allergic reaction can also lead to anaphylactic shock, urticaria, IgE-dependent swelling, or some forms of bronchial asthma. This type reaction is typical of allergies of genetic origin, IgE-dependent, known as atopic.

**Type 2** allergic reaction (cytotoxic) develops when allergisation is accompanied by the appearance of specific antibodies, usually from IgG or IgM class. The antibodies react with allergens on the surface of cell membranes (usually blood cells or bone marrow cells), which consequently undergo decomposition.

**Type 3** allergic reaction takes place when allergen reacts with IgG, IgM, IgA immunoglobulin class antibodies or IgE forming immune complexes, which deposit in tissues initiating inflammations. Type 3 reaction is often caused by drugs, foreign species serum, food products and organic or inorganic dust.

**Type 4** allergic reaction is known as a delayed or cellular reaction. The main role in this type is played by lymphocytes T allergised by a specific allergen, excreting cytokines triggering inflammatory reaction in tissues. Recent studies have indicated the possibility of the involvement of immunoglobuline E in the type 4 reaction [33, 52].

GENERAL CHARACTERISATION OF ALLERGENS

Allergens are divided into those originating from the natural environment and those originating from a chemically contaminated environment. In the first group, the most frequent are the airborne allergens occurring in pollen of different plants, mould fungi spores and in fragments of mycelial hyphae. The airborne allergens also include: bacteria, house dust mites, epidermis of house pets, allergens of some food products and insect venom. The allergens originating from the natural environment are usually proteins, being high-molecular compounds of molecular weight higher than 10 kDa. The allergens originating from a chemically contaminated environment are usually low-molecular chemical compounds and elements of the nature of heptanes. They acquire complete antigen properties only after linking with proteins in an organism. Their best known representatives include: metals, drugs, additives to food products, latex, aldehydes [33, 52].

Pollen allergens are water-soluble proteins or glyco-proteins of molecular weight from 10–70 kDa [3, 13, 18, 40, 48, 50, 65, 95]. Many pollen allergens are resistant to pH changes and to high temperature, even up to 100°C [50]. Their nomenclature has been unified on the basis of recommendations published by the World Health Organisation in 1994. The designations are derived from the first three letters of the genus and the first letter of the species. The Arabic numeral accompanying the letters denotes the sequence of identification and description of a given allergen, e.g. the allergens of *Ambrosia artemisiifolia* are named as Amb a 1, Amb a 2, Amb a 3 [39]. Until 1994, the allergens were also named following this rule, but in italics and with Roman numerals, e.g. the allergens of *Artemisia vulgaris* were referred to as Art v I, Art v II, Art v III [46, 48, 65, 95].

Apart from the pollen grains, antigens can also be present in the other parts of plants in roots, stems, leaves, seeds, fruit, which has been proved in e.g. *ragweed*, *grass,*
and plantain [25, 42, 53, 59]. Allergic reaction can also be triggered by some substances excreted by plants, such as juice and volatile oils, or in other bioaerosols of plant origin, e.g. fluids released during treatment of some plants (crop, cotton, herbs) [15, 42]. It has been established that one allergen is farnezol occurring in the volatile oil excreted by flowers of the lime tree [80]. The pollen grains of mugwort, also contains, apart from proteins, sesquiterpenes stimulating allergic reactions, which cause airborne contact dermatitis [64].

### CROSS REACTIONS

Proteins of different antigens show certain analogies in the sequence of amino acids determining immunological similarity and cross-reactivity. The cross reactions take place between the antibody and the allergen of a chemical structure similar to that of the antibody whose production it stimulates [33, 58]. The major allergens of the pollen of trees growing in the temperate climatic zone are structurally and immunochemically similar. In the species of the same genus the repeatability of the amino acid sequences reaches 80-90% [48, 94]. For example, cross reactions are observed between allergens of grass pollen within one genus, and between the genera [18, 50, 54, 88], between the antigens of birch and ash tree pollen [69, 70, 86], within the family Fagaceae, Corylaceae and Betulaceae [50, 75], and between the antigens of pollen of some trees, herbal plants and fruit, as well as edible vegetables [14, 40, 59, 60, 89, 91, 94].

### CHARACTERISATION OF SELECTED POLLEN ALLERGENS

**Hazel (Corylus spp.).** The major allergen of hazel pollen is Cor a 1 of the molecular mass 13.5 kDa. Although the hazel pollen count is moderate to low (20–30 grains in 1 m³), the pollen reveals strong allergenic properties, enhanced by the fact that it can enter into intense cross-reactions with the antigens of the pollen of birch and alder trees [39, 40, 50, 75].

**Alder (Alnus spp.).** The main allergen in alder tree pollen is Aln g 1 of the molecular mass 17 kDa [39, 50]. Its allergenic properties are related to high concentrations in the atmosphere, reaching even above 2000 grains in 1 m³ [90], and the possibility of cross-reactivity with birch pollen. Fortunately, in the period of alder tree pollination the exposure to its pollen is limited because low temperatures in February and March do not favour long outdoor presence [75].

**Birch (Betula spp.).** Birch pollen reaches a very high concentration in air, reaching 5000 grains in 1 m³ [9, 16, 90]. Allergy symptoms can also occur on contact with house dust in which the maximum concentration of birch pollen is observed in the three weeks after the peak of pollination [27, 63]. The major allergen of birch pollen is Bet v 1 of the molecular weight 17 kDa, composed of 159 amino acids. It enters into the cross-reaction with the apple allergen of the same molecular weight. Bet v 2 has 133 amino acids in a sequence close to that of the proteins from the prophilline group taking part in polymerisation of actine. Bet v 3 of the molecular weight 20 kDa is the kallmodulin, which activates many enzymes [39, 50]. Bet v 7, identified by Cadot et al. [8], has the molecular mass of 18 kDa and belongs to cyclophilines characterised by high cross-reactivity with the other birch pollen antigens. Over 90% of people suffering from pollinosis are allergic to the allergens of birch pollen [9]. In 1972, Belin, using immunochimical methods, demonstrated that antigens leave the birch pollen grain within one minute of contact with a solution of the physiological saline, and proved that the majority of allergens leave the pollen grain in the first 45 minutes of contact with physiological salt. Grote et al. [25, 26] localised the allergy-inducing proteins in the birch pollen grain as occurring mainly in the cytoplasm on amyloplasts, in sporoderm and near the pores.

**Grasses (Poaceae).** Allergens of grass pollen are the most common cause of allergic inflammation of nasal mucosa and conjunctivitis. The concentration of grass pollen grains in air reaches the very high values of 4000–5000 grains in 1 m³. In Holland and France, as many as 80% of pollinosis sufferers are allergic to antigens of grass pollen [88]. Allergens of particular grass species show intense cross-reactivity [18, 88]. The allergy-inducing proteins from grass pollen have been comprehensively studied and on the basis of the structural similarities have been divided into 7 groups. Group 1 comprises glycoproteins of the molecular weight of 27 kDa. They are localised in sporoderm, in the vicinity of starch grains and in cytoplasm of the pollen grain. The best known allergen is Lol p 1. Groups 2 and 3 comprise non-glycosylcic proteins of the molecular weight of 11 kDa, and the amino acids sequence close to that of Lol p 1, the allergens of group 4 have the molecular weight of 57 kDa, together with those of group 5–30 kDa. The allergens of group 6 are cytichromes, and those of group 7 are prophillines of the molecular weight 14 kDa [50, 60, 89]. The allergenic properties of prophillines are related to the degree of air pollution. In the highly industrialised areas and in big cities they are the major allergens, and in other areas they are the minor allergens [83]. Prophillines can be responsible for the cross-reactions between the allergens of grass pollen and the allergens of edible vegetables [59]. Masuch et al. [47], studying the allergy-inducing properties of Lolium perenne, have shown that the presence of ozone increases the concentration of the allergen Lol p 5 in the pollen grains of this grass. Schoene et al. [71] have found that in the presence of ozone the number of pollen grains of Lolium perenne containing amyloplasts decreases and vacuolization of the grains with amyloplasts is stronger.
Sorrel (Rumex spp.). The species of the Rumex genus are characterised by high pollen production as an individual plant can produce about 400 million grains. Despite this high number of grains, its allergen is not particularly clinically important [20]. Horak and Jäger [30] found allergic reaction to the antigens of sorrel in 20% of pollinosis sufferers. Maasch and Geissler [44] have proved that as many as 70% of pollinosis patients have positive reaction to the Rumex allergens - result of the cross-reaction between the allergens of the pollen of grass and sorrel.

Mugwort (Artemisia spp.). The antigen activity of the Artemisia pollen is high and its allergens are responsible for the majority of pollinosis symptoms observed in late summer [37]. According to Wallenstein and Kersten [87], and Spieksma [74], 3–10% of pollinosis sufferers are allergic to the Artemisia allergens, the majority of which are also allergic to the pollen of grass, ragweed and the allergens of apple and celery [26, 28, 50].

The concentration of mugwort pollen just above the ground level is several times higher than at a height of 15–25 m [77]. The major allergens of mugwort are Art v 1 of the molecular mass 60 kDa and Art v 2–20 kDa [48, 76].

Plantain (Plantago spp.). The concentration of plantain pollen in air reaches low to moderate values. The allergies to its allergen are accompanied by allergies to pollen of other plant species. On the basis of studies performed in many European cities [43, 73] the concentration of plantain pollen in air has been found to be low and irregular. In the period of plantain pollination, Bryant et al. [5] reported (from Sydney) that the patients developing asthma symptoms were simultaneously allergic to the plantain allergens. The allergy to plantain allergens was noted in 84% patients with asthma.

As the peak of pollination occurs in June, the allergic symptoms can be mistakenly associated with grass pollen allergy [29].

Ragweed (Ambrosia spp.). Ragweed pollen is a common cause of pollinosis in North America. Until the end of the 60s, it was believed that ragweed did not occur in Europe, and therefore had not been considered as a source of serious allergenic threat. In recent years, ragweed appeared in Europe in hitherto unknown localities, and the number of people allergic to the allergens of this plant has been gradually increasing [45, 61, 79].

In Europe, the ragweed pollen has a non-uniform distribution. It is abundant in Hungary, Ukraine and northern Italy, less frequent in France, the Balkan Peninsula, Switzerland, Austria, Slovakia and the Czech Republic [34, 36, 45, 92]. In 1993, about 20% pollinosis sufferers were allergic to Ambrosia pollen, while in 1997 their number increased to about 70% [93]. Horak et al. [31] have shown that high concentrations of the ragweed sporomorphs in the air in Vienna have been introduced via long-distance transportation. Similar phenomena have been observed in many European countries [12, 34, 61, 78].

The ragweed allergens have been thoroughly recognised and the amino acid sequence has been determined for Amb a 1/2, Amb a 3, Amb a 5, Amb a 6 and Amb t 5 [39, 48, 50]. Ambrosia pollen cross react with almost all other composites, especially with mugwort pollen. Further cross reactions are known with dandelion, goldenrod, sunflower and chamomile [12].

Pinaceae (Pinaceae). Allergenic activity of the pollen of Pinaceae is low [21, 32]; however, its allergens can cause allergic reaction [55, 72]. The concentration of pine pollen in air reaches 8000 grains in 1 m³ [23]. The pollen may be important as adjuvant enhancing the symptoms triggered by grass pollen allergens [55, 72].

FACTORS CONDUCIVE TO ALLERGY TO POLLEN

Genetic factors

Inherited susceptibility to allergies depends on many genes. The processes affected by inherited features include: regulation of immunoglobulin E production, enhanced capability of activation of cells involved in allergic reactions, release of histamine and production of cytokines and oversensitivity of tissues or organs affected by allergic reaction [52]. If both parents have the same pollinotic symptoms the probability of allergy to pollen in their child is of about 80% [49].

Environmental factors

Air pollution. Increasing air pollution in industrialised areas and big cities with ozone, formaldehyde, sulphur dioxide, nitrogen oxides, and industrial dust increases the risk of allergies [10, 13, 38, 51, 66].

Ozone has an irritating effect on the airways mucous membrane. It is formed as a result of complex photochemical reactions between the volatile organic substances, nitrogen oxides and sunlight. These reactions are particularly intense in big cities in summer after the morning rush hour. Kehrl et al. [38], Spieksma [74] and Riediker et al. [62] have proved a sensibilising effect of ozone in people suffering from asthma of allergic origin. Formaldehyde irritates the respiratory tract, impairing the ciliary apparatus, which in turn facilitates the access of allergens to the tissues. It is released as a gas from paints, lacquers, glues, carpets, floor coverings, insulating materials and furniture made of chipboards [64]. Sulphur dioxide is formed as a result of combustion of high-sulphur-content coal. Nitrogen oxides come mainly from exhaust gases, especially diesel exhausts, and gas cookers. These compounds can damage the mucous membrane of the respiratory tract, cause an increase in the mucus
viscosity, irritate the endings of nerve fibres in bronchi, and thus can trigger inflammation. They have also been found to occur on the surface of pollen grains [10, 62].

According to a recent study, many more pollinosis sufferers live in the cities than in the country [4, 6, 7, 11, 51, 67].

Exposure to allergens. People with atopy (predisposed to type I allergies) should try to avoid prolonged contact with large doses of the allergen. In the cities, between tall buildings, the air circulation is disturbed, which can lead to an increased level of pollen counts in the area [17]. People working in certain professions (farmers, orchard growers or greenhouse workers) are periodically exposed to very high pollen counts, which may be related to the increased incidence of pollinosis [15].

Apart from an effective pharmacological therapy, people suffering from allergies should be informed about the mechanisms of allergies and methods of avoiding exposure to allergens in order to improve their quality of life. The threshold values of pollen count at which clinical symptoms can develop are given in Table 1.

Infections of the respiratory tract. Virus infections, particularly in children, can stimulate an allergic inflammatory response [29] and cause the exacerbation of allergic disease [52].

Diet. Everyday diet includes increasing amounts of processed food, conserved food products and products containing leavening agents, stabilisers, artificial dyes and sulphur dioxide. In agriculture, there is an increasing use of pesticides, nitrogen fertilisers, antibiotics and hormones in animal production. These chemicals contain many agents inducing allergy [14].

Microflora of pollen grains

While airborne, pollen grains interact with different substances suspended in air, including microorganisms that deposit on the pollen grain surface [11]. According to Schäppi et al. [68], these interactions can change properties of pollen grains.

A mixed microflora, consisting of bacteria and fungi, is present on the surface of pollen grains of anemophilous plants. Most probably the pollen grains are infected at the moment of pollination by microorganisms originating from the plant surface. It cannot be excluded that some allergic symptoms appearing as a result of exposure to pollen are enhanced by the microorganisms and endotoxins on the pollen grains that can act as adjuvants [81].

REFERENCES


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<th>Taxon</th>
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<td>20</td>
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Table 1. Threshold values of pollen count (at which allergy symptoms develop).


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Characterisation of pollen allergens


