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Pollens as allergens

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SUMMARY. After a brief description of the allergenic pollens involved in pollinosis in Italy, the author outlines the structure of the pollen grain and its mechanism of allergenicity. The methods of isolating and evaluating allergens are listed, in addition to the principal allergenic pollens. In conclusion, the author underlines the need for international standardization of allergenic extracts to be used for research and clinical purposes.

Key words: allergen, cross-reactivity, immunological properties, pollen.

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Pollinosis is the most common allergic disorder. As is well known, the clinical manifestations of pollinosis are determined by a specific hypersensitivity to pollen, especially anemophilous pollens of certain plants, among genetically susceptible individuals.

Pollen is indispensable to the reproduction of seed-bearing plants (Spermatophyta) in that it represents the male gametophyte, whose task is to fertilize the omospecific ovules. It should be noted, by the way, that a complex genetic code exists also among vegetables. According to this code the protein antigens of pollen grains contain special markers by which they are recognised and accepted only by species-specific receptors on the pistils, thus preventing any incompatible fecundation (Pacini, 1990). Not all pollens cause allergy nor are all equally allergenic. We may regard as strictly allergenic those species of plants which trigger disturbances in a significant number of subjects over large areas (Spieksma, 1990). This means about twenty plants.

In Italy, certain plants are notoriously allergenic: Poaceae, *Parietaria*, some Compositae, while others are considered to be rather less so: Chenopodiaceae, Plantaginaceae, Polygonaceae. As for trees, the most allergenic are: *Olea*, Betulaceae, Corylaceae, some Coniferae (Cupressaceae, Taxodiaceae), some Fagaceae, Fabaceae, Salicaceae and Platanaceae (Ciampolini and Cresti, 1981; D'Amato, 1981; De Leonardis *et al.*, 1985; Negrini and D'Amato, 1985; D'Amato *et al.*, 1988; Negrini, 1989; Errigo, 1990).

On this subjects, it is worth remembering the postulates so clearly defined by Thommen in 1931 whereby a pollen may be able to sensitize an atopic subject and thus bring on clinical symptoms:

1) it must contain an antigenic component capable of inducing sensitivity;

2) it must belong to an anemophilous plant;

3) it must be produced in large quantities;

4) it must be light enough to be carried long distances;

5) it must belong to a plant which grows widely.

Such conditions are undoubtedly fulfilled in the vast majority of cases.

Under exceptional conditions, pollens from entomophilous plants (some Asteraceae, Acacia dealbata among Fabaceae, etc.) can also give rise to sensitization, for instance as a result of high dispersion in air or direct handling of the plant in flower (Spieksma, 1990).

The first of Thommen's postulates listed above is particularly important: the pollen must contain a specific component which causes sensitization (antigen) and which is specifically able to induce an IgE-mediated antibody response (allergen); it can then bind to such antibodies, thus potentially triggering clinical symptoms. This may explain the relatively low incidence of pollinosis caused by anemophilous plants, in relation to the large number of such plants.

A clear example is provided by Pinaceae *(Pinus)*, *Castanea*, *Fagus*, *Quercus* spp. and others. Although in some localities these plants are widely found, they do not very often determine an allergic sensitization; this can probably be explained by the low level of allergenicity of their pollens (Spieksma, 1990).

While pollens constitute the classic source of allergens, several studies have revealed the presence of antigens in various parts, such as the roots, stem and leaves of some plants, e.g. *Ambrosia*, Poaceae, and *Plantago* (Rebhun *et. al.*, 1954; Baldo *et al.*, 1982; Burge, 1989). A collation of the results of recent research into the immunochemical determination of airborne allergens with the pollen count shows a good correlation between the two curves, though frequently with a wider range of values for the airborne allergens. This indicates that the allergens come not only from the pollen grain but also from other parts of the plant.

Studies by Solomon *et al.* (1983) on *Ambrosia* and by Spieksma *et al.* (1990) on Graminaceae have confirmed the presence of airborne allergenic determinants in fractions of submicron dimensions, though the clinical role of these allergens remains to be defined (Pan *et al.*, 1990).

Without going into the details of the structure of the pollen grain, it is worth mentioning some of its features: the endoplasmatic reticulum of the cytoplasm, which is responsible for protein synthesis; the Golgi complex for carbohydrate synthesis; the mitochondria for the metabolic needs of the cell; the storage granules containing starch, proteins and lipids; and the nucleus, which is equipped with chromatin and nucleoli. The intine, which is present in all pollens, originates from the cytoplasm and wraps the granule internally. It is made up of cellulose, pectin-like substances, proteins and enzymes.

The exine, which is structured in two layers (intesine, internally and ectesine, externally) is mainly made up of sporopollenin, which seems to be formed by the oxidative polymerization of carotenes and esters of carotenes (Brooks and Shaw, 1968).

The material obtained from aqueous extraction of pollen yields complex mixtures of proteins and glycoproteins, pigments, carbohydrates, low molecular weight (dialyzable) substances, etc., among which there are many antigens including the allergen(s) responsible for clinical symptoms. Pollen allergens are contained in the internal layer (intine) of the granule and emerge through pores on the surface of the granule.

When pollens come into contact with the moist conjunctival surface, of the nose and throat, their antigenically active protein or glycoprotein fractions are eluted. Some of the enzymes contained in the intine, and which are

among the first proteins to be released by the pollen granule, might enable allergens to penetrate the mucosa more easily, thus facilitating sensitization (Bousquet and Michel, 1979). The dynamics of the release of the allergenic fractions was neatly demonstrated by Belin and Rowley in 1971. On incubating *Betula* granules with antipollen allergen antiserum and using immunofluorescence, these authors found considerable quantities of allergens outside the granules within a short time of incubation. This shows the speed with which the phenomenon takes place, just as in the case of human nasal mucosa. Moreover, when the same authors used the simple radial immunodiffusion technique on *Betula* pollen grains placed in agar containing anti-*Betula* antiserum, they observed that the antigen-antibody precipitation bands formed outside each of three pores in the membrane of the grains, thus showing that the antigens must be located mainly in the intine. Furthermore, the presence of allergens also in the exine has recently been confirmed by immunocytochemical research and by transmission electron microscopy (Cerceau, 1990).

Appropriate studies based on suitable techniques are essential in order to identify the allergenic components of pollens, to define their activity and to standardize them for laboratory purpose and for diagnostic and therapeutic purposes.

Various fractions isolated from pollen extract are immunogenic and hence antigenic. Only a few of these, however, have allergenic properties. According to the degree of reactivity shown by sensitized individuals towards these components, allergens can be classified as «major» or «minor». «Major» allergens may be defined as those which trigger a reaction in the majority of allergic patients (90% according to Marsh's studies (1975) on allergopathic genetics, by means of the prick test; 50% according to Løwenstein's studies (1980) using CRIE) and towards which half of these patients present specific IgE with a marked binding ability. «Minor» allergens are those which, though contained in the same extracts, have a marked effect only on a small number of patients (10% or fewer) or a mild effect on a large number of patients.

Within the general structure of proteins or glycoproteins, pollen allergens usually have a molecular weight between 5,000 and 60,000 Daltons (mainly 10,000 - 30,000); they are therefore not dialyzable (a higher molecular weight hinder penetration of the mucous membrane). They generally have an acidic isoelectric point. Many pollen allergens are resistant to heat treatment at 100° C and to marked variation in pH (Baer *et al.*, 1988). By contrast, it is worth mentioning Amb a I (of *Ambrosia artemisiifolia* or *elatior*) as an exception to the heat resistance of pollen allergens.

Another point which should be stressed is the possibility of cross-reactivity between different substances which have the same group of antigenic determinants (epitope) in common. Cross-reactivity between pollen antigens of different genera of the same family has indeed been observed: concerning Poaceae, for instance, cross-reactivity has been noted between antigens of group I-II-III of *Lolium perenne* and those of *Dactylis glomerata*, *Phleum pratense*, *Festuca elatior*, and *Holcus lanatus* (Løwenstein and Østerballe, 1986). Cross-reactivity has been demonstrated between pollens of the Oleaceae *(Olea europaea*, *Fraxinus excelsior*, *Ligustrum vulgare*, and *Phillyrea angustifolia)* (Bousquet *et al.*, 1985).

Within the Betulaceae family, cross-reactivity exists between *Alnus* and *Betula*, and within the Corylaceae family between *Corylus* and *Carpinus*. The phenomenon has also been documented between different genera of these families and to a lesser extent among Betulaceae, Corylaceae and Fagaceae (Weber, 1981; Negrini *et al.*, 1987).

A partial cross-reactivity also exists between some trees and vegetable foodstuffs (Eriksson *et al.*; Van Toorenenberger, 1990): a frequent finding in pollinosis caused by Betulaceae is hypersensitivity to certain fruits (apple, pear, peach, apricot, cherry, walnut, hazelnut, banana) and to vegetables such as fennel and carrot, giving rise to a characteristic Oral Allergic Syndrome which is often accompanied by symptoms in other region of the body (Ortolani *et al.*, 1988). In some pollinosis caused by herbaceous plants too, cross-reactivity with vegetable foodstuffs has been observed. Such a link can be seen between allergy to Compositae *(Artemisia)* and hypersensitivity to celery; between Gramineae and melon, tomato, orange, water-melon, kiwi; between *Ambrosia* and melon and banana; and between *Parietaria* and mulberry (Ortolani, 1989; Von Toorenenbergen, 1990).

The process of identification, isolation and purification of pollen allergens begins with the extraction and solubilization of the pollen collected from blossoms in aqueous buffers. The aqueous extract then undergoes various analyses according to the aim of the research.

Regarding the chemical composition, the principal methods used involve the determination of the protein content, total nitrogen content and protein-bound nitrogen and subsequently the analysis of the amino-acid structure, glucides, lipids, etc. As for the physical properties (molecular weight, electrical charge, isolectrical point) the methods applied are: analytical ultracentrifugation, high pressure liquid chromatography (HPLC), electrophoresis in polyacrylamide gel containing sodium dodecylsulfate (SDS-PAGE), electrophoresis (on acrylamide, agarose, etc.) and isoelectric focusing (IEF). Immunological techniques for antigen characterization involve immunoprecipitation methods such as immunodiffusion (ID), immunoelectrophoresis (IEP) and crossed-immunoelectrophoresis (CIE). Finally, in order to define the real allergenic properties and biological potency, pools of sera from ascertained subjects are used in cross-radioimmunoelectrophoresis (CRIE), immunoblotting, RAST and RAST-inhibition test, leukocyte histamine release and skin test (Vicari, 1986; Baer *et al.*, 1988; Marsh *et al.*, 1988; Younginger, 1989). The use of monoclonal antibodies as monospecific reagents in some of the above immunological techniques has recently been suggested (Baldo, 1986; Jäger *et al.*, 1989). Equally recently, the immunochemical techniques have been backed up by recombinant DNA techniques, which also enable the aminoacid sequences to be identified and produced more symply than by chemical purification methods («files» of cDNA from *Betula* and *Ambrosia* allergens have recently been compiled) (Klapper and Rafnart, 1989; Pettenburger *et al.*, 1989).

Without wishing to list all the allergenic pollens which have been identified and purified, we should mention here a few of the principal ones, following the nomenclature recommended by the IUIS special commission (Aas, 1980; Marsh *et al.*, 1988; Marsh *et al.*, 1989; Geraci and Cocchiara, 1990).

Poaceae

Lolium perenne: 5 main allergens have been isolated (Lol p I-II-III-IV and Lol p X, where Lol p I represents the «major» allergen, shown to be active in over 95% of patients tested); molecular weight ranges from 11,000 to 56,800 Daltons.

Phleum pratense: from the 28 or so antigens, 11 allergens have been isolated, among which the 4 main ones have been purified and characterized (Phl p V-VI-VII and VIII); MW ranges from 10,00 to 34,000 Daltons.

Dactylis glomerata: 13 allergens, among which Dac g I has been purified, MW of 33,000 Daltons.

Poa pratensis: 2 allergens, the main one being Poa p X with MW of 12,00 Daltons; it does not contain carbohydrates.

Asteroideae

Ambrosia artemisiifolia (elatior): 6 main allergens, defined as Amb a I-II-III-IV-V-VI, with molecular weights ranging from 5,000 Daltons (Amb a V) to 38,000 Daltons (Amb a I, formerly called Antigen E, and Amb a II); these latter are the «major» allergens.

Ambrosia trifida: the main allergen is Amb t V with MW of 4,390 Daltons.

Urticaceae

Parietaria judaica: at least 25 antigens have been identified, 9 of which have allergenic properties. Four of these may be classified as «major» allergens; the main one to be purified and characterized (Par j I) has MW of 10,000 Daltons, while the other 3 have MW of 20,000; 30,000 and 40,000 Daltons respectively.

Parietaria officinalis: of the 20 or so antigens, 4 have been found to possess allergenic properties, the main one being Par o I with MW of 14,000 Daltons. Cross-reactivity between allergens of *Parietaria judaica* and *Parietaria officinalis* is typical in patients allergic to these plants; no cross-reactivity is found between *Parietaria* and *Urtica dioica.*

Betulaceae

Alnus incana: the main allergen, Aln i I, has MW of 22,500 Daltons.

Betula verrucosa: the main allergen, Bet v I, has MW of 17,000 Daltons. The aminoacid composition of this allergen bears a strong resemblance to that of Aln i I of *Alnus incana,* which suggests that these two allergens are structurally and immunologically homologous.

Corylaceae

Corylus avellana: main allergen Cor a I, with MW of 13,500 Daltons.

Oleaceae

Olea europaea: from among at least 16 antigens, one «major» allergen (Ole e I), with MW of about 17,000 Daltons; a second allergen (Ole e II), of about 10,000 Daltons and a third, with MW greater than 50,000 Daltons, have also been identified.

In conclusion, we should stress the need for a standardized titration of allergenic activity for all extracts used for clinical, diagnostic, therapeutic and research purposes. To this end, a Subcommittee for Allergen Standardization has been set up by the International Union of Immunological Societies (IUIS) and is recognised by the WHO. Its task is to oversee the preparation of reference samples (International Standards) and to provide directives for their use by the various laboratories. To date, reference samples have been registered for the pollens of *Phleum pratense, Ambrosia elatior, Betula, Cynodon dactylon* and *Lolium perenne,* while those for *Parietaria, Artemisa vulgaris, Olea* and *Quercus* are under way. It is to be hoped that these indispensable standards will become available as soon as possible.

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