

Allergens/antigens

CHAPTER 27

Aerobiology of pollen and fungal allergens

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Clinically important pollinosis is primarily due to flowering plants with wind-dispersed pollens. Although these anemophilous species form a minority of flowering plants, they produce prodigious amounts of pollen. Entomophilous (insect-pollinated) plants produce relatively few pollen grains. Plants having only a portion of their pollen grains airborne are termed amphiphilous. Their role in pollinosis is not clear.

The pollen-producing organs, stamens, consist of an anther attached to a filament. Allergenic pollens develop in and are released from pollen sacs contained in the anthers. Pollen sacs are lined with a nutritive cell layer, the tapetum. During its formation, each immature pollen grain secretes about itself a clear layer, the intine. Around this the tapetum deposits a multilayer exine composed of a polymer, sporopollenin. Release of pollen from the anther is known as anthesis. Drying creates gaps in the anther wall through which pollen escapes. Airborne pollen levels tend to increase during warm, dry, clear conditions and fall during cool, moist periods. Although pollen grains can travel several hundred miles, concentrations of windborne pollens generally decrease sharply within a few hundred meters of their source.

Intact pollen grains are presumed to be the primary carriers of allergen, but ragweed allergens have been associated with particles $<5\text{ }\mu\text{m}$, and *Amb a I* (antigen E) activity has been found in submicronic particles. Pollen allergens can be extracted from the somatic portion of ragweed plants and may become airborne in vegetable debris. Furthermore, allergens might be eluted from pollen grains deposited on moist surfaces, with dispersion of the resultant extract in droplets.

Air sampling relies on recovery of pollen grains for microscopic evaluation. The gravity (Durham) sampler uses greased microslides for pollen collection. This obsolete device is limited by uncertainty in the volume of air sampled and a tendency to

underrepresent smaller particles severely. More exacting goals require volumetric devices, such as the rotating-arm impactor (Rotorod; Ted Brown Associates, Los Altos Hills, Calif.), that relate recoveries to index volumes of air. Silicone and Lubri-Seal grease are preferred adhesives for impactor surfaces. These collectors calculably underrepresent small particles, and during prolonged sampling the particle load reduces collecting efficiency. Wind-oriented spore (Burkard) traps (Burkard Manufacturing Ltd., Hertfordshire, England) are among the most versatile collectors for a variety of particles, including pollens. These devices also are especially useful for collecting fungal spores and other agents well below $10\text{ }\mu\text{m}$. Samplers should be placed on an unobstructed rooftop. Sampling closer to the ground sometimes reveals exposures not reflected by rooftop samplers. Personal collectors using small filters and compact pumps can determine individual exposure patterns. High-volume samplers with fiberglass filters have been used to collect aerosols that can be extracted for immunochemical analysis. This sampling may provide a better overall view of total allergen exposure.

Most airborne pollens range in size from 12 to $70\text{ }\mu\text{m}$. Certain features make identification easier (e.g., air bladders on coniferous pollens). Some grains have a smooth exine (psilate); others have characteristic projections coalescing to form spines (echinate). Most demonstrate apertures called pores when rounded or furrows when elongated. Grains may have only pores (porate), only furrows (colpate), or both—usually with pores in furrows (colporate). Pores may be capped by an operculum (e.g., grass pollens) or be surrounded by a refractile shield (aspis) (e.g., birch).

Trees and other woody plants are the earliest to undergo pollination each growing season. Tree pollination especially varies in date and intensity. Nonwoody perennial species (e.g., grasses) generally follow the trees from late spring to midsummer. In southern and west coast areas, grasses may pollinate throughout much of the year. Anemophilous annuals (e.g., ragweed) generally shed pollen in midsummer

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to late summer, depending on their seed germination dates.

Establishing a dose-response relationship between pollen exposure and symptoms is difficult. The range of severity for individuals is quite broad, and symptoms often reflect concurrent exposure to several allergens. Response usually increases with ongoing short-term exposure (priming), and exposure involves aerosol fractions besides intact pollen grains. Pollens usually penetrate to the level of the glottis. Most deposit in the nose, pharynx, esophagus, stomach, and eyes.

The protein molecules in pollens capable of sensitizing patients generally range in size from 10,000 to 40,000 daltons. Once the pollen grain is in contact with the upper airway mucous membranes, symptoms develop within a few minutes, suggesting that these proteins are rapidly eluted. Although individuals are exposed to many potentially allergenic pollens, only a relatively few pollens produce symptoms. This relative restriction of sensitization reflects the broad patterns of cross-reactivity among proteins derived from different but taxonomically related plants.

Methods for assessing cross-reactivity among pollen extracts may be divided into approaches that measure all proteins or antigens (e.g., gel diffusion against animal antisera, isoelectric focusing, and crossed immunoelectrophoresis [CIE]) and those that examine the allergenic relationships of the extracts (e.g., RAST inhibition and crossed radioimmunoelectrophoresis [CRIE]). RAST inhibition cannot demonstrate to what extent individual allergens in two pollen extracts are related or unique. CRIE, although less quantitative than RAST, offers the advantage of separately analyzing each allergen. These methods demonstrate considerable cross-reactivity within and between many plant families.

For the Cupressaceae, cedars, cypresses, and junipers are important in pollinosis; other conifers have only minor allergenic significance. Marked cross-reactivity occurs within the cypress family, with little cross-reactivity extending to other conifers. Partially purified mountain cedar pollen allergens in the 40,000 to 50,000 dalton range have been described and a purified allergen (*cry j* I) has been obtained from Japanese cedar.

The Fagaceae (beech) and Betulaceae (birch) families produce pollens that strongly cross-react. Birch species appear to be the most closely related to each other. Immunotherapy for one may be adequate to treat allergy to others and may also be sufficient to treat allergy to beech family members.

The Salicaceae (willows, poplars, and aspens) strongly cross-react with each other and show some

degree of cross-reactivity with the birch and beech families. Oleaceae (olive, ash, and privet) show strong cross-reactivity. Reports of cross-reactivity in the Aceraceae (maple and box elder) are conflicting.

Of the Poaceae, the subfamily Festucoideae contains most of the grasses important in causing allergic symptoms in the northern United States. These grasses demonstrate extensive cross-reactivity. For most allergic patients, treatment and testing may be conducted with one or a few members of this family. Purified *Lolium* (rye) allergens have been obtained, termed *Lol p* I, II, and III (formerly rye I, II, and III). Eighty-five to 95% of patients exhibit reactivity to the grass group I allergens (*Lol p* I and its homologs). A subfamily, Eragostoideae, contains one predominant allergenic member, Bermuda grass. The subfamily Panicoideae is represented by Bahia grass and Johnson grass in the southern United States. Separate testing and treatment is necessary for Bermuda, Bahia, and Johnson grasses because of their low degree of cross-reactivity with the Festucoideae.

In the Urticaceae and Asteraceae families, nettle and pellitory show a lack of cross-allergenicity. A 12,000 dalton allergen has been purified from pellitory. Among the composites, major allergens have been identified from the tribe Ambrosieae. The most important is *Ambrosia artemisiifolia* (short ragweed) allergen *Amb a* I (antigen E). Other purified antigens include *Amb a* II (antigen K), *Amb a* III (Ra3), *Amb a* V (Ra5), and *Amb a* VI (Ra6). Several other allergens have been partially characterized. Some antigenic differences exist between giant and short ragweed species. In areas where Asteraceae other than ragweed are clinically significant, adequate immunotherapy will not be achieved by using ragweed extracts alone if pollen levels are significant.

The Chenopodiaceae and Amaranthaceae families contain major inducers of pollinosis in the western United States. An antigen from Russian thistle (*Sal p* I) has been isolated. Members of the *Chenopodiaceae* are closely related and cross-react strongly with the amaranth family. Russian thistle, however, appears to contain potent allergens (some of which are unique) that require special treatment. Russian thistle may provide relevant materials for some of the other chenopods, such as lamb's quarters, but generally chenopods of local significance require individual therapy.

FUNGAL ALLERGENS

Most human serum contains at least some fungus-specific IgG, and specific IgE to several fungi has been detected in atopic patients. Fungi reproduce by

simple fragmenting of the fungus body (mycelium) or by highly specialized cells (spores) produced on fruiting branches. Three classes of fungi are of primary concern; Zygomycetes (e.g., *Mucor*, *Rhizopus*), Ascomycetes (e.g., powdery mildews, brewer's yeast), and Basidiomycetes (e.g., rusts, smuts, and most mushrooms). A fourth class, Deuteromycetes ("fungi imperfecti"), is an artificial grouping of asexual fungal stages. *Aspergillus*, *Penicillium*, and *Alternaria*, which for years were considered "imperfects," are now known to be asexual stages (form genera or form species) of Ascomycetes. Many fungi have two names because the sexual and asexual stages were described separately. Furthermore, fungal nomenclature may undergo repeated changes; for example, what was formerly *Hormodendrum* is now called *Cladosporium*. Because many fungi produce morphologically different sexual and asexual spores that become airborne, describing symptom-exposure relationship becomes difficult.

Fungi can grow on a variety of substrates, provided certain temperature and moisture conditions are met. Thus exposure to fungal material is inescapable in any outdoor, frost-free environment. The most abundant airborne asexual fungi (*Cladosporium*, *Alternaria*, *Epicoccum*, *Stemphylium*, *Curvularia*, *Torula*, *Aspergillus*, and *Penicillium*) represent this category of fungi. Many fungi, especially *Aspergillus* and *Wallemia*, grow actively in house dust, having adapted to very dry environments. High prevalence areas exist in which organic material is abundant.

When seasonal temperatures fall below freezing, spore concentrations significantly decrease due to decreased spore production; snow cover further decreases the level by preventing dispersal of previously formed spores. Indoors, mold growth and spore levels are directly related to relative humidity in the range of 30% to 70%. Below 30% humidity there is little interior mold growth.

Spores are released into the air by either mechanical disturbance or active discharge mechanisms. During hot, dry afternoons in the Midwest, high levels of *Alternaria*, *Cladosporium*, and *Epicoccum* predominate. A rainstorm will wash spores from the air, and rain-splash fungi (yeasts) and biologically dispersed ascospores (*Letposphaeria*) increase. Basidiospores that can be discharged without precipitation are abundant in early morning hours. Once airborne, spore clouds travel long distances. Smaller spores, which settle out slowly, require high wind speeds for impaction.

Fungal spores have a wide variety of shapes and a broad size range, from <2 to >100 μm . Many cannot be identified without associated fruiting structures.

Impaction samplers (Rotorod) are relatively efficient for fungal spores greater than 10 μm . However, smaller spores, including those of all basidiomycetes, many ascospores, and important form genera such as *Cladosporium*, *Aspergillus*, *Penicillium*, and all yeasts, are not efficiently sampled; losses may be as high as 90%. Common spores that are readily recovered and identified using these methods include *Alternaria*, *Epicoccum*, *Stemphylium*, and *Curvularia*.

Suction sampling is the method of choice for spores in general. By combining volumetric samples with culture methodology, another group of fungi can be enumerated: those that are readily grown and identified on artificial medium. This group partly overlaps the category with identifiable spores and also includes *Aspergillus*, *Penicillium*, yeasts, and a variety of other asexual fungi and ascomycetes. However, culturing has limitations. Many fungus colonies inhibit the growth of other fungi within a distance of several millimeters. Furthermore, some spores are not living at the time of sampling. Finally, other fungi, although theoretically capable of growth, require highly specific substrates. Even when ideal sampling methods including culture are used, most of the ascospores and basidiospores that are present in the air cannot be specifically identified.

Alternaria spores appear to contain antigens that are distinct from mycelial antigens. Evidence exists that spore-derived antigens provoke a higher rate of skin reactivity and provide more valid in vitro results by RAST. However, pure spore material is not readily available. Many commercial fungal antigen preparations are made from fungus culture material with no spores and only indirect association with mycelium. These products, although producing relatively high rates of skin reactivity, may not contain antigens relevant to allergic symptoms; in fact, they cross-react only weakly with spore or mycelium-derived antigens. Many fungi produce variable numbers of spores from week to week in continuous culture. Thus each fungus varies with respect to amounts and kinds of potential antigens produced. Additional factors may contribute to batch-to-batch differences in allergen potency of extracts prepared serially from single strains.

Several fungal allergens have been purified, including *Alt a* I (a major allergen of *Alternaria alternata*), a carbohydrate-rich protein of about 30,000 daltons. Two allergens, *Cla h* I and *Cla h* II, have been isolated from *Cladosporium herbarum*. Patterns of cross-reactivity also occur in fungal species. Within *Aspergillus*, the *fumigatus* and *niger* groups appear antigenically distinct from the *versicolor/nidulans/glaucus* group. *Alternaria*, *Stemphy-*

lium, *Curvularia*, and *Stachybotrys* cross-react extensively in in vitro systems, and an *Alt a 1*-like allergen appears to be as abundant in *Stemphylium* as *Alt a 1* is in *Alternaria*.

Aerobiology of pollen and fungal allergens

Which of the following statements is/are true regarding air sampling devices?

- The gravity slide (Durham) sampler underrepresents smaller particles
- Prolonged sampling runs will diminish the collection of pollens by the Rotorod impactor method
- Wind-oriented spore (Burkard) traps are the most efficient available devices for collecting agents below 10 μm for microscopic analysis
- Samplers are best placed at ground level to assess the overall (background) intensity of pollen exposure in a community
- Suction samplers are most efficient in outdoor air when the entrance orifice is stationary (i.e., in a fixed position)

Explanation: Gravity slide samplers are most successful in collecting larger, relatively numerous pollen types. The collecting properties of adhesive coatings used on Rotorod samplers diminish with prolonged sampling times. Wind-oriented suction traps are substantially more efficient in collecting fungal spores and agents $<10 \mu\text{m}$ than are other sampling devices. Sampler devices must be placed in unobstructed areas, away from building edges at heights of 50 feet above the ground to achieve optimal "average background" levels. Suction devices must be continually oriented to the wind to minimize collection errors.

Answer: a, b, and c are true.

Reference: Solomon WR. Aerobiology of pollinosis. J ALLERGY CLIN IMMUNOL 1984;74:449-61.

Factors that tend to diminish the quantity of ragweed pollen emission and/or airborne dispersal include:

- Nighttime temperatures above 50° F
- Ground fog at sunrise
- Prolonged frontal rains
- Low relative humidity
- Increasing lengths of darkness

Explanation: Anthesis and aerial pollen dispersal is favored by low relative humidity. Ground fog indi-

cates high humidity. Prolonged frontal rain effectively "washes" pollen grains from the air, whereas brief thunderstorms, due to turbulent airflow, may briefly enhance pollen dispersion by refloating deposited grains. Anthesis is depressed at temperatures below 50° F. Flowering of short ragweed requires a specific period of nighttime darkness; thus shorter days favor pollination.

Answer: b and c.

Reference: Solomon WR. Aerobiology of pollinosis. J ALLERGY CLIN IMMUNOL 1984;74:449-61.

Methods that effectively detect cross-allergenicity among pollen extracts include:

- Isoelectric focusing
- CIE
- RAST inhibition
- CRIE

Explanation: Isoelectric focusing separates proteins in pollen extracts by isoelectric point independent of allergenic activity. CIE demonstrates the number of antigens in an extract to which animal (rabbit) antibodies can be raised. Neither method detects IgE binding (allergenicity). RAST inhibition can be used to quantitate cross-allergenicity but cannot demonstrate to what extent individual allergens in two extracts are shared, related, or unique. CRIE offers the advantage of analyzing individual allergens separately.

Answer: c and d.

Reference: Weber RW, Nelson HS. Pollen allergens and their interrelationships. Clin Rev Allergy 1985; 3:291-318.

Using collections from volumetric suction samples, spores from the following fungi can be related microscopically to their specific form genus:

- Aspergillus*
- Alternaria*
- Penicillium*
- Cladosporium*
- Curvularia*

Explanation: Depending on the experience of the observer, several fungal spores have distinct morphologic features by which they can be identified. These include *Cladosporium*, *Alternaria*, *Epicoccum*, *Stemphylium*, *Curvularia*, *Fusarium* (macrospores), *Leptosphaeria*, *Chaetomium*, *Pleospora*, *Ganoderma*, *Coprinus*, and a variety of rusts and smuts. The large

proportion of airborne spore types including *Aspergillus* and *Penicillium* are spheres smaller than 10 μm that have no distinguishing characteristics. Although their biologic nature may be evident, such fungi can be identified, on occasion, by culture techniques.

Answer: b, d, and e.

Reference: Burge H. Fungus allergens. Clin Rev Allergy 1985;3:319-29.

On a hot, dry, breezy day in the midwestern United States, the pattern of fungal spores contained in the air would include high levels of:

- a. *Epicoccum*
- b. *Leptosphaeria* species
- c. *Aspergillus*
- d. Basidiospores
- e. *Cladosporium*

Explanation: *Epicoccum*, *Alternaria*, *Cladosporium*, and smut spores are considered "dry spores," which predominate in atmospheric samples obtained during warm, dry weather. Rain will increase the number of "splash fungi" (e.g., yeasts) and actively dispersed ascospores (e.g., *Leptosphaeria* species). *Basidiomyces* are abundant in predawn and early morning when heavy dew accumulates. *Ganoderma*, a Basidiomycete, sporulates even during dry days. *Aspergillus* and *Penicillium* predominate indoors.

Answer: a and e.

References: Burge H. Fungus allergens. Clin Rev Allergy 1985;3:319-29.