Guidelines for using pollen cross-reactivity in formulating allergen immunotherapy

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There is a trend among allergen extract companies to diminish the number of individual extracts available for commercial use. This is driven in part by economic considerations and in part by constraints imposed by the US Food and Drug Administration. As much as 2/3 of previously manufactured extracts are or will no longer be available. In this setting, knowledge of pollen allergen interrelationships and cross-reactivity is crucial for formulation of inhalant allergen immunotherapy. In addition, as optimal doses for major allergens become clarified, cross-reactivity considerations should also affect formulation. Adverse reactions because of overdosing can occur if cross-reactivity is ignored.

Plant systematic (taxonomy) is becoming more precise. The taxonomy of Judd et al[1] is supported by morphologic, embryologic, and palynologic evidence, as well as analysis of biochemical, chloroplast gene, chromosome, and DNA data. Cloning of recombinant allergens has identified homologous proteins and clarified functions. It has been suggested that protein family content, or molecular classification, rather than botanical taxonomy is a superior way to address cross-reactivity issues.[2] However, as has been shown with profilins and major grass allergens, even a high degree of amino acid homology does not always mean strong cross-reactivity.[3]

Two premises govern cross-reactivity inferred by plant systematics. The first is that accepted botanical taxonomy reflects phylogeny: that 2 plants in the same genus evolved from a common progenitor, and 2 in the same family or order did so from more distant ancestors. The second premise is that closely related plants will have a greater number of shared antigens than distantly related ones. This approach has been generally validated. The exception is there are many highly preserved proteins, even across plant, fungi, and animal kingdoms, that share cross-reactivity. Examples are profilins, lipid transfer proteins, and pathogenesis-related proteins. The small differences that exist between these ubiquitous, presumably vital proteins explain why these are frequently, although not always, only minor allergens.

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Vascular plants (Tracheophytina) include spore-forming ferns and club mosses, and seed plants; the latter group traditionally is split into Gymnosperms and Angiosperms (flowering plants). Monophyly of angiosperms is strongly supported, but that of the Gymnosperms is not, although in the latter, cycads, gingkoes, and conifers are closely related. The division of Angiosperms into Monocotyledonae and Dicotyledonae is likewise not supported by recent data: monocots are monophylous, but dicots are not. A large related subset is the tricolpates, also known as true dicots (eudicots), which has been identified by a pollen type containing 3 apertures, and similar gene sequences.

On the basis of these data, certain recommendations can be made concerning allergen extract choices for diagnosis and immunotherapy.[4] Such recommendations are meant as guidelines only, to be modified by regional factors such as floral prevalence. Where related plants appear to have adequate cross-reactivity and extracts have similar potency, it appears wise to use the locally most prevalent member. The following discussion uses botanical groupings and incorporates the findings into guidelines for extract formulation.

CONIFERS

Many conifer allergenic vaccines can be freely switched with each other. The older literature showing interchangeability of extracts within the Cupressaceae family is well supported by recent allergen characterization. Strong cross-allergenicity within the family is a result of marked homology of group 1 and 2 major allergens. Such members include *Cupressus*, *Juniperus*, *Cryptomeria*, and *Chamaecyparis*. Therefore, a single member, perhaps *Juniperus ashei*, would provide adequate coverage in extract formulation. Pine family members in Pinaceae, such as pines and spruces, if clinically relevant, need to be treated separately. Weak cross-allergenicity between conifers and angiosperms may be a result of group 4 calcium-binding proteins, or other pan-allergens such as prolifin, and is usually not clinically important.

GRASSES

The grass family Poaceae is the largest of the Monocots and the most relevant to allergy. Within the Pooideae subfamily of northern pasture grasses, cross-allergenicity is strong because of marked homology of major allergen groups 1, 2/3, and 5. This is a very large group including several tribes with members such as brome, fescue, ryegrass, June, timothy, orchard, sweet vernal, velvet, and canary grasses, as well as cereal grains. Older research with crude extracts suggests possible unique allergens in timothy and sweet vernal. Generally, 1 of 2 members should suffice for treatment, and timothy has been shown to be a very adequate

TABLE I. Recommended representatives for allergen immunotherapy

Family/subfamily	Scientific name	Common name	Comments
Cupressaceae	*Juniperus ashe*	Mountain cedar	Strong cross-allergenicity within family on the basis of marked homology of group 1 and 2 major allergens
Taxodioideae	*Cryptomeria japonica*	Japanese red cedar	
Pinaceae	*Pinus strobus*	White pine	No cross-reactivity with Cupressaceae
Poaceae	*Phleum pratense*	Timothy	Strong cross-allergenicity on the basis of marked homology of group 1, 2/3, and 5 major allergens
Pooideae			
Chloridoideae	*Cynodon dactylis*	Bermuda	Lack of group 2 and 5 allergens accounts for little cross-allergenicity with Pooideae
Panicoideae	*Sorghum halepense*	Johnson	
Sapindaceae	*Acer negundo*	Box elder	Disparity between *A negundo* and other *Acer*
	Acer rubrum	Red maple	
Urticaceae	*Urtica dioica*	Nettle	Little cross-reactivity
	Parietaria judaica	Pellitory	
Betulaceae	*Betula verrucosa*	Silver birch	Strong cross-allergenicity between Betulaceae members on the basis of homology of group 1 and 2 major allergens
	Alnus glutinosa	European alder	
Fagaceae	*Quercus alba*	White oak	Strong cross-allergenicity between Betulaceae and Fagaceae on the basis of homology of group 1 and 2 allergens
Amaranthaceae	*Amaranthus retroflexus*	Redroot pigweed	Strong cross-allergenicity between *Amaranthus* species
	Atriplex canescens	Wingscale	Strong cross-allergenicity between *Atriplex* species
Chenopodioideae	*Kochia scoparia*	Burning bush	Chenopodioideae members show greater diversity, with variable degrees of reactivity
	Salsola pestifer	Russian thistle	Russian thistle may possess unique allergens
Oleaceae	*Olea europaea*	Olive	Strong cross-allergenicity between Oleaceae members because of homology of group 1 allergens
	Fraxinus excelsior	European ash	Choose dominant local species
Asteraceae	*Ambrosia artemisiifolia*	Short ragweed	Strong cross-allergenicity between short, giant, western, and false ragweed
	Ambrosia trifida	Giant ragweed	
	Artemisia vulgaris	Mugwort	Strong cross-allergenicity between *Artemisia* species
	Artemisia tridentata	Giant sagebrush	Choose dominant local species
	Iva xanthifolia	Burweed marshelder	Minor to little cross-reactivity between ragweed and mugwort, marshelder, or cocklebur
	Xanthium communis	Cocklebur	

choice to represent the subfamily. Use of additional members may be appropriate for optimal concentration of allergen groups, and consider sweet vernal where it is prevalent. Otherwise, June or perennial ryegrass are good choices.

The Panicoideae and Chloridoideae subfamilies have greater diversity, and the lack of groups 2 and 5 allergens in both of these groups accounts for decreased cross-reactivity with northern pasture grasses. Members of these tribes should be tested for and treated separately from Pooideae. The former contains Johnson, Bahia, corn, and sugarcane. Chloridoideae contains Bermuda, lovegrass, and prairie grasses such as salt, buffalo, and grama; cross-reactivity is seen between members, with Bermuda the strongest inhibitor. Therefore, Bermuda is the appropriate choice to cover these other prairie grasses. Panicoideae does appear to show more cross-reactivity with Pooideae than Chloridoideae. Grass allies, such as palm or sedge, if felt to be clinically relevant, need to be treated separately. Although such monocots demonstrate cross-allergenicity within families, there is no cross-reactivity with Poaceae. The cross-reacting profilins and calcium-binding proteins are minor allergens of grasses and of little practical consequence in vaccine formulation.

TREES AND WEEDS (TRICOLPATE ANGIOSPERMS)

Diversity is the rule among tricolpate angiosperms, and lack of cross-reactivity is the rule down even to the level of tribe. There are exceptions, with significant cross-reactivity across certain family boundaries. This large clade contains both trees and weeds. Although allergists are used to thinking of trees and weeds separately as early-season and late-season pollinators, respectively, there are early pollinating weeds and fall pollinating trees. Botanically, several families contain both trees and shrubs or weedy members, and in terms of cross-allergenicity, it is best not to segregate artificially on the basis of size.

In Amaranthaceae family weeds, *Amaranthus* and *Atriplex* genera show strong cross-allergenicity among members of the same genus and can each be represented by single members. However, the other locally relevant family members need to be addressed separately. Greater diversity, with variable degrees of reactivity, exists within subfamily Chenopodioideae members, and Russian thistle, *Salsola pestifer*, possesses unique allergens and needs to be treated separately. The other major tumbleweed, burning bush, *Kochia scoparia*, likewise is best treated separately.

The order Fagales shows strong cross-allergenicity within the birch family, Betulaceae, and extending to the beech family, Fagaceae. Use of the locally prevalent Betulaceae member, frequently birch or alder (*Betula* and *Alnus*, respectively), should cover other family members as well. In areas where oaks are predominant, *Quercus* would be expected to cover birch. With other members of the Rosidae subclass, generally 1 member of a family can be expected to be adequate for immunotherapy, although there is a scarcity of data and there are exceptions such as the Urticaceae members, nettle and pellitory (*Urtica* and *Parietaria*, respectively), which share no significant cross-allergenicity.

Oleaceae members are strongly cross-reactive: in areas where European olive, *Olea europaea*, is grown, it seems the proper choice, whereas in areas where ash, *Fraxinus*, is prevalent, it should be adequate. Asteraceae members show variable cross-reactivity. The 4 major ragweeds are short, giant, western, and false (*Ambrosia artemisiifolia*, *Ambrosia trifida*, *Ambrosia psilostachya*, and *Ambrosia acanthicarpa*, respectively). They strongly cross-react, and 1 should be quite adequate for therapy, although common use is a mix of short and giant ragweed. In areas where the sages, *Artemisia*, or marshelders, *Iva*, are common, these need to be handled separately. Because *Artemisia* species are strongly cross-allergenic, it is not necessary to skin-test or treat with multiple members. In the Midwest and West states, choose common sagebrush, *Artemisia tridentata*, whereas in the eastern states and Europe, mugwort, *Artemisia vulgaris*, seems most appropriate.

CONCLUSION

Refinement of plant systematics with clarification of phylogenetic relationships has demonstrated that certain basic premises hold true: cross-allergenicity does mirror taxonomy in the very great majority of cases, with more distantly related plants showing fewer shared allergenic proteins. Clarification of allergen groups in important aeroallergenic plant sources explains earlier clinical observations as well as cross-reactivity experiments with crude allergen extracts. It is hoped that these observations, summarized in this article and in Table I, provide a rational structure for decisions of extract formulation.

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