Evaluation of cross-reactivity of allergens by use of intradermal testing in atopic dogs

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Objective—To examine cross-reactivity of aeroallergens in Colorado and surrounding states by evaluating concurrent positive reactions of related and nonrelated allergens of intradermal tests in dogs.

Sample Population—Intradermal test results of 268 atopic dogs.

Procedure—A retrospective evaluation of skin test results for 268 dogs was performed. Pairs of closely related and nonrelated allergens were evaluated. Group 1 consisted of closely related allergens with demonstrated antibody cross-reactivity in humans. In group 2, allergens of the same plant group (ie, trees, grasses, or weeds) that were not closely related were paired. In group 3, allergen pairs were of different plant groups. Plant allergens were paired with dust mite allergens, animal dander, or mold spores in group 4. In the last group, allergens not derived from plants were paired. Data were evaluated twice by use of a different definition of a positive reaction. Significance of the difference between group means of log odds ratios was estimated by use of a bootstrap percentile confidence interval.

Results—Significant differences in the number of concurrent positive reactions were not found between related versus nonrelated grass, weed, or tree allergens. Significant differences in the number of concurrent positive reactions were found between plant allergens of different groups (ie, grasses, weeds, and trees) and plant allergens of the same groups, related or nonrelated , as well as between plant-derived and nonplant-derived allergens. Many dogs reacting to a specific allergen did not react to a closely related allergen at the same time.

Conclusion—These results provide evidence against clinically relevant cross-reactivity and suggest that allergen-specific immunotherapy should be formulated on the basis of single allergen test results. (*Am J Vet Res* 2002;63:874–879)

Canine atopic dermatitis is one of the most common diseases encountered in small animal practice.[1-3] It is characterized by pruritus and secondary infections and diagnosed by history, physical examination findings, and the exclusion of differential diagnoses such as scabies or adverse food reaction by appropriate procedures such as skin scrapings or elimination diets.[1,3-5] Glucocorticoids are used commonly to treat atopic dermatitis; however, the frequent occurrence of adverse effects with regular glucocorticoid treatment and the increased public awareness about these potential adverse effects have dramatically increased the willingness to administer alternative treatments. Antihistamines and essential fatty acids have been successful in decreasing or eliminating the clinical signs of atopic dermatitis in a small group of dogs.[6-9] Allergen-specific immunotherapy is another safe and effective long-term treatment modality.[10-13] To formulate an allergy vaccine, allergens are selected on the basis of the results of intradermal testing or serum testing for allergen specific IgE. In humans, cross-reactivity of IgE antibodies to related allergens, particularly various grass pollens, was demonstrated by evaluating skin tests,[14] Prausnitz-Kuestner extinction,[15,16] Ouchterlony gel double immunodiffusion,[17,18] and radioallergosorbent test inhibition.[19-22] On the basis of this documented cross-reactivity, mixes of grass pollen allergens have been used in place of single allergens in in vitro tests as well as in the formulation of allergen-specific immunotherapy in human medicine to decrease costs and increase efficacy. This research was extrapolated to small animals, and in vitro and in vivo tests using allergen mixes based on available human data are offered.

Evidence for cross-reactivity of allergen-specific IgE antibodies against related allergens in veterinary medicine is lacking. A recent Australian study[23] looking for evidence of cross-reactivity of antibodies against related allergens in canine atopic dermatitis was based on 1,000 skin tests and failed to document a difference between the occurrence of concurrent positive reactions to closely related plant allergens and nonrelated plant allergens, indicating that antibody cross-reactivity may not be pronounced in canine atopic dermatitis. The aim of the study presented here was to examine cross-reactivity of aeroallergens in Colorado and surrounding states by evaluating concurrent positive reactions of related and nonrelated allergens of 268 intradermal skin tests in dogs

Materials and Methods

Intradermal tests of 268 atopic dogs from Colorado and the surrounding states were evaluated retrospectively. Atopic dermatitis was diagnosed by consistent history, compatible clinical signs, and ruling out differential diagnoses such as food adverse reactions or scabies with appropriate diagnostic methods or therapeutic trials.[1] On the basis of results of studies evaluating cross-reactivity of allergen-specific IgE antibodies in humans and on affiliation to the same genus or family, 5 groups of allergen pairs were formed. Group 1 con-

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Table 1—Single and concurrent intradermal tests results in dogs, using selected plant allergen pairs

Allergen 1		Allergens 1 & 2		Allergen 2	
Name	No. of positive reactions	OR*	No. of positive reactions	No. of positive reactions	Name
Orchard grass	51	76.8	39	52	Perennial rye grass
Meadow fescue	57	9.7	34	62	June grass
Short ragweed	91	60.1	76	99	Western ragweed
Western water hemp	91	36.8	69	95	Rough pigweed
Maple	79	12.9	64	111	Box elder
Dermatophagoides pteronyssinus	108	3.2	79	153	*D farinae*
Perennial rye grass	52	7.8	31	62	Bermuda grass
Meadow fescue	57	12.2	36	62	Bermuda grass
Short ragweed	91	15.4	65	93	Prairie sage
Giant ragweed	89	38	74	96	Marsh elder
River birch	66	26.7	51	78	Pine
Mountain cedar	74	36.7	61	83	American elm
June grass	62	14.3	44	74	Olive
Mountain cedar	74	11.5	55	94	Lamb's quarter
Bermuda grass	62	10.6	46	91	Short ragweed
Perennial rye grass	52	8.8	39	94	Lamb's quarter
Red top	28	13.8	23	83	American elm
Pine	78	18	59	87	Plantain

OR = Odds ratio.
*An OR of 1.0 indicates no association; an OR > 1.0 indicates positive association; an OR < 1.0 indicates a negative association.
Positive reaction defined as a 2, 3, or 4+ reaction (evaluation A).

Table 2—Single and concurrent intradermal tests results in dogs, using selected allergen pairs containing allergens not derived from plants

Allergen 1		Allergens 1 & 2		Allergen 2	
Name	No. of positive reactions	OR*	No. of positive reactions	No. of positive reactions	Name
Feathers	29	3.3	14	66	River birch
Perennial rye grass	52	3.9	27	78	*Alternaria* spp
Red top	28	1.4	18	153	*Dermatophagoides farinae*
American elm	83	4.2	49	108	*D pteronyssinus*
Cat epithelium	67	4.4	36	78	*Helminthosporium* spp
Cladosporium spp	70	28.2	58	87	Plantain
Feathers	29	1.8	20	153	*D farinae*
Cat epithelium	67	2.7	38	108	*D pteronyssinus*
Alternaria spp	78	1.6	50	153	*D farinae*
Cladosporium spp	70	11.2	56	108	*D pteronyssinus*
Feathers	29	4.1	17	78	*Helminthosporium* spp
Cat epithelium	67	4.8	37	78	*Alternaria* spp

See Table 1 for key.

sisted of 6 pairs of closely related allergens with demonstrated antibody cross-reactivity in humans and included perennial rye grass (*Lolium perenne*) and orchard grass (*Dactylis glomerata*),[20] meadow fescue (*Festuca elatior*) and June grass (*Poa pratensis*),[19,20,24] short ragweed (*Ambrosia elatior*) and Western ragweed (*Ambrosia psilostachia*),[19,21] Western water hemp (*Amaranthus tuberculatus*) and rough pigweed (*Amaranthus retroflexus*),[14,25] box elder (*Acer negundo*) and maple (*Acer rubrum* and *A saccharum*), and the house dust mites *Dermatophagoides farinae* and *D pteronyssinus*. Allergens in group 2 were chosen from the same group of plant pollens (grasses vs weeds vs trees), and allergens not closely related with a lack of cross-reactivity in human patients were paired. Pairs consisted of Bermuda grass (*Cynodon dactylon*) and perennial rye grass,[19,20] Bermuda grass and meadow fescue,[19,20] prairie sage (*Artemisia tridentata*) and short ragweed (*Ambrosia elatior*),[21] marsh elder (*Iva xanthifolia*) and giant ragweed (*Ambrosia trifida*),[21] pine (*Pinus* ssp) and birch (*Betula nigra*), and mountain cedar (*Juniperus ashei*) and American elm (*Ulmus americana*).[19] In group 3, individual grass, weed, or tree pollens were paired with an allergen from another plant group. Pairs consisted of June grass and olive (*Olea europaea*), mountain cedar and lamb's quarter (*Chenopodium album*), Bermuda grass and short ragweed, perennial rye grass and lamb's quarter, red top (*Agrostis alba*) and American elm, and pine and English plantain (*Plantago lanceolata*). Plant allergens were paired with dust mite allergens, animal dander, or mold spores in group 4. Pairs consisted of birch and feathers, *Alternaria* spp (fungi) and perennial rye grass, red top and *D farinae*, American elm and *D pteronyssinus*, *Helminthosporium* spp (fungi) and cat dander (*Felis catus*), and *Cladosporium* spp (fungi) and English plantain. In the last group (group 5), allergens not derived from plants were included. Pairs consisted of *D farinae* and feathers, *D pteronyssinus* and cat dander, *Alternaria* spp and *D farinae*, *Cladosporium* spp and *D pteronyssinus*,

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Table 3—Single and concurrent intradermal tests results in dogs, using selected plant allergen pairs

Allergen 1		Allergens 1 & 2		Allergen 2	
Name	No. of positive reactions	OR*	No. of positive reactions	No. of positive reactions	Name
Perennial rye	18	190.6	12	21	Orchard grass
June grass	14	93.4	8	16	Meadow fescue
Short ragweed	31	402.5	26	37	Western ragweed
Rough pigweed	25	80.5	17	31	Water hemp
Maple	18	21.2	9	32	Box elder
Dermatophagoides pteronyssinus	43	4.9	24	129	*D farinae*
Bermuda grass	14	118	9	18	Perennial rye
Bermuda grass	14	56.7	8	16	Meadow fescue
Short ragweed	31	35.7	13	31	Prairie sage
Giant ragweed	28	61.2	16	29	Marsh elder
Pine	9	51	3	10	River birch
American elm	18	36.5	11	24	Mountain cedar
Olive	13	100.4	8	14	June grass
Mountain cedar	24	33.1	14	26	Lamb's quarter
Bermuda grass	14	158	12	31	Short ragweed
Perennial rye	18	43.9	10	26	Lamb's quarter
American elm	18	98	13	19	Red top
Pine	9	16.3	3	20	Plantain

See Table 1 for key.
Positive reaction defined as a 3 or 4+ reaction (evaluation B).

Table 4—Single and concurrent intradermal tests results in dogs, using selected allergen pairs containing allergens not derived from plants

Allergen 1		Allergens 1 & 2		Allergen 2	
Name	No. of positive reactions	OR*	No. of positive reactions	No. of positive reactions	Name
Feathers	6		0	10	River birch
Alternaria spp	10	4.6	2	18	Perennial rye
Red top	19	1.4	8	129	*Dermatophagoides farinae*
American elm	18	0.3	1	43	*D pteronyssinus*
Helminthosporium spp	11	3.5	2	20	Cat epithelium
Cladosporium spp	10	4.1	2	20	Plantain
Feathers	6	1.6	2	129	*D farinae*
Cat epithelium	20	0.6	2	43	*D pteronyssinus*
Alternaria spp	10	2.2	5	129	*D farinae*
Cladosporium spp	10	1.6	2	43	*D pteronyssinus*
Feathers	6		0	11	*Helminthosporium* spp
Alternaria spp	10	1.6	1	20	Cat epithelium

See Table 1 for key.
Positive reaction defined as a 3 or 4+ reaction (evaluation B).

feathers and *Helminthosporium* spp, and cat dander and *Alternaria* spp. All dogs were tested with each of these allergens. Evaluations of the data were performed twice, with a positive reaction being defined as at least 2+ in evaluation A and at least 3+ in evaluation B.

Statistical evaluation—Evaluations of skin test results are typically performed by comparing the reactions to a positive control (typically histamine) graded as a 4+ and a negative control graded as 0.[1,4,26] Evaluation of the data was performed with a positive reaction being defined as at least 2+ (evaluation A) or 3+ (evaluation B). For each pair of allergens considered, the degree of association between the positive responses was estimated by use of the **odds ratio**. The odds ratio is the odds of a positive response for allergen 1, among those having a positive response for allergen 2, divided by the odds of a positive response for allergen 1, among those having a negative response for allergen 2. An odds ratio of 1.0 indicates no association, an odds ratio > 1.0 indicates positive association, and an odds ratio < 1.0 indicates a negative association. To compare groups, the logarithm (base *e*) of each odds ratio was computed, and the mean of the logs was computed for each group. Using logarithms makes the responses more normally distributed and reduces the influence of unusually high values for individual comparisons on the group mean.[27]

Significant differences between group means of the log odds ratio were estimated by use of a bootstrap percentile confidence interval.[28] Bootstrap replications of size n = 268 were randomly selected with replacement from the data. The difference between the group means of log odds ratios was computed for each of the 2,000 bootstrap replications. The (p/2) and 1 – (p/2) quantiles of the 2,000 differences were then used as the upper and lower limit of a 2-sided 100p% confidence interval. If the value 0 was at the edge of a 2-sided 100% confidence interval, then the *P* value assigned was p. More sophisticated versions of the bootstrap are available; however, this method was judged to be adequate because of the large sample size and the approximate normality of the log odds ratio. When there are no concurrent positives for 2

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Table 5—*P* values for comparisons of the group mean log OR for different groups of allergen pairs

	Mean log OR		*P* values*							
			Group 2		Group 3		Group 4		Group 5	
Groups	A	B	A	B	A	B	A	B	A	B
Group 1	3.00	4.13	0.834	0.810	0.036	0.690	< 0.001	< 0.001	< 0.001	< 0.001
Group 2	2.97	4.00	NA	NA	0.037	0.841	< 0.001	< 0.001	< 0.001	< 0.001
Group 3	2.53	4.06	NA	NA	NA	NA	< 0.001	< 0.001	< 0.001	< 0.001
Group 4	1.52	0.674	NA	NA	NA	NA	NA	NA	0.166	0.322

*$P < 0.05$ is considered significant.

Group 1 consisted of pairs of closely related allergens with demonstrated antibody cross-reactivity in humans. Group 2 consisted of pairs of allergens of the same plant group (ie, trees, grasses, or weeds) that were not closely related with a lack of cross-reactivity in humans. Group 3 consisted of pairs of allergens of different plant groups. Group 4 consisted of plant allergens that were paired with dust mite allergens, animal dander, or mold spores. Group 5 consisted of pairs of allergens that were not derived from plants..

A = Evaluation A, in which a positive reaction was defined as at least 2+ out of 4+. B = Evaluation B, in which a positive reaction was defined as at least 3+ out of 4+

NA = Not applicable.

allergens, the estimated odds ratio is 0, and the logarithm of the odds ratio is undefined. When that occurred, a missing value code was inserted, and the means were computed without that number.

Results

Concurrent reactions of different pairs and their odds ratios were determined (**Table 1–4**), as were the *P* values of the individual comparisons of the mean log odds ratios (**Table 5**). According to evaluation B, there was no significant difference in the number of positive concurrent reactions between closely related plant allergen pairs and plant allergen pairs not related. In evaluation A, there was a significant difference in the number of positive concurrent reactions between pairs of nonrelated pollen allergens of different plant groups (trees, grasses, and weeds) versus either related or nonrelated pollen allergens paired within the same group of grasses, weeds, and trees. There was a significant difference in the number of positive concurrent reactions between plant allergens of groups 1, 2, and 3 and allergens belonging to other major groups such as animal epithelia, house dust mites, or molds with both evaluations. No significant difference was found in the number of positive concurrent reactions between groups 4 and 5 with both evaluations, in which various allergens of nonplant origin were involved. Evaluating the closely related allergens in group 1, 25 and 35% of the dogs with a strong reaction to an allergen did not react to the paired closely related allergen in evaluation A and B, respectively.

Discussion

Allergen-specific immunotherapy is based on results of intradermal testing or serum testing for allergen-specific IgE. If allergen mixes are part of the diagnostic test used, and a positive reaction is observed to such an allergen mix, all allergens involved are included in the extract used for immunotherapy. A positive reaction to an allergen mix without involvement of all allergens of the mix in the disease process is possible and leads to administration of irrelevant allergens to the patient. Administration of subcutaneous injections of allergen extract to clinically normal dogs increased skin reactivity to these allergens.[29] However, another study[30] failed to show induction of positive intradermal reactions by immunotherapy with irrelevant allergens. Similar studies performed in atopic patients are lacking. Dogs with a predisposition to develop atopy had been sensitized by injections of allergens and subsequently developed clinical atopic dermatitis.[31] These studies did not conclusively demonstrate the potential of injections of allergens not involved in the atopic dermatitis of a dog to induce allergic reactions in the patient (if allergens in these mixes are not antigenically similar). To the authors' knowledge, no such study evaluating the effect of injecting nonrelevant allergens on clinical disease in atopic dogs has been performed. However, the possibility of clinical deterioration cannot be excluded and should concern the clinician treating dogs with allergen-specific immunotherapy.

Cross-reactivity of IgE antibodies against different allergens in human medicine was initially suspected on the basis of frequent concurrent skin test reactions of allergic individuals to 2 related allergens.[14] Concurrent positive reactions to 2 allergens may not always indicate cross-reactivity of involved allergen-specific IgE antibodies but may be the result of concurrent true hypersensitivity to 2 allergens without any cross-reactivity. True cross-reactivity may only be detected by use of other techniques and was demonstrated in human medicine by Prausnitz-Kuestner extinction,[15,16] Ouchterlony gel double immunodiffusion,[17,18] and radioallergosorbent test inhibition.[19-22] However, a positive reaction to 1 allergen and a concurrent negative reaction to the other allergen are evidence against cross-reactivity of IgE against these 2 allergens.

Skin test reactivity was used as evidence of IgE cross-reactivity against conifer pollens in humans.[32] More evidence of true cross-reactivity in human medicine was presented via inhibition and absorption studies.[19-21,24,33,34] These studies revealed considerable cross-reactivity between antibodies to perennial rye grass, June grass, orchard grass, and meadow fescue and poor cross-reactivity between that group and IgE against Bermuda grass and sweet vernal.[20] A similar study[19] duplicated these results. In this particular study, inhibition was also performed with different ragweeds and revealed a clear cross-reactivity of IgE antibodies against short, giant, false, and western ragweed. A

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complete lack of allergenic association between a conifer (mountain cedar, *J sapinoides*) and trees belonging to the angiosperms such as oak (*Quercus alba*), beech (*Fagus grandifolia*), and elm (*U americana*) was noted.[19]

More recently, a number of major antigens of various grass, weed, and tree pollens and house dust mites have been isolated. A detailed review of the literature is beyond the scope of this article. However, what seems to emerge is a concept of some shared antigens between various pollens, particularly grass pollens and highly individual antigens, often in addition to the former. A similar situation seems to be present with various mites, including house dust and parasitic mites. In veterinary medicine, antigens are not characterized well, and studies evaluating cross-reactivity of allergen-specific IgE indirectly (assessing concurrent skin test reactions) or directly (inhibition studies) are rare. Noli et al[35] provided the first evidence that dust mite allergens relevant to human medicine are not as important to veterinary dermatology. A major allergenic fraction of *D farinae* in dogs was isolated and characterized recently.[36] The 2 components of this fraction differ from the major characterized antigens of *D farinae* in humans. A recent publication[37] characterized and cloned a major dust mite allergen for dogs, Der f 15, which is different from major allergens defined in human medicine so far. To the authors' knowledge, no such studies have been published in regard to pollen or mold antigens in veterinary medicine.

Evaluation of concurrent skin test reactions is an indirect method of assessing cross-reactivity of allergen-specific IgE antibodies. As discussed, it is not precise in documenting this cross-reactivity. However, the lack of concurrent reactions is reliably indicating a lack of cross-reactivity of involved antibodies. Evaluation of intradermal test reactions with a grade of 0 to 4, compared with a positive and negative control, is used by most veterinary dermatologists.[1,3,4,26,37] This evaluation is subjective. Evaluation of the data was therefore performed twice with a positive reaction being defined as at least 2+ in evaluation A and at least 3+ in evaluation B.

Concurrent positive reactions in the closely related allergens in our study are not significantly different from concurrent reactions in unrelated plant allergens. Depending on the definition of a positive reaction, a concurrent positive reaction to a closely related allergen was not present in approximately 1 in 4 (evaluation A) or 1 in 3 (evaluation B) dogs with positive reactions to an allergen, which is evidence against cross-reactivity occurring to a relevant degree in canine atopic dermatitis. Differing skin test reactivity may not exclude the possibility of shared individual epitopes that may only be detected in inhibition and absorption studies. However, these shared epitopes do not lead to a similar degree of mast cell degranulation and release of inflammatory mediators, as measured by intradermal testing. Thus, their clinical relevance is questionable on the basis of the current understanding of canine atopic dermatitis. These results suggest that immunotherapy should be formulated on the basis of single allergen testing to avoid exposure to allergens not involved in the dog's atopic disease. However, with evaluation A there was a significant difference between the mean log odds ratio of nonrelated pollen allergen pairs from different plant groups (trees vs grasses vs weeds) and those from grass, tree, or weed pollen allergen pairs, either closely related or nonrelated. One possible explanation is the existence of common epitopes on all tested grass pollens (or weed or tree pollens) similar to what has been found in human medicine. This difference was not present with evaluation B. That may be the result of the decreased ability of the shared epitopes to cause mast cell degranulation in intradermal testing, compared with individual epitopes.

There was a significant difference in the number of concurrent positive reactions between plant-derived allergens (related or nonrelated) and mold-derived, dust-mite-derived, and animal-derived allergens. This difference could be the result of different lifestyles of the dogs with subsequent exposure to different allergens and development of hypersensitivities against the allergens to which it was predominantly exposed. Another explanation could be the presence of a plant-derived antigen common to most pollens, which triggers hypersensitivity to plant pollens in the dogs' environment more readily than reactions to other allergens. Further studies using inhibition and absorption techniques are needed to evaluate the degree of cross-reactivity in the dog.

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