Stability and mixing compatibility of dog epithelia and dog dander allergens

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Background: Little information or data are available concerning the stability and compatibility of dog epithelia and dog dander allergens.

Objective: To determine the immunochemical reactivities of commercial, nonstandardized dog epithelia and dog dander extracts after exposures to various temperatures or after mixing with high-protease fungal and cockroach extracts at concentrations recommended for maintenance immunotherapy (IT) injections.

Methods: Quantitative enzyme-linked immunosorbent assay and qualitative (immunoblot) analyses were performed to compare specific compositional changes with total or individual allergen activities. Assays for dog allergens Can f 1 and Can f 3 (albumin) used specific mouse or rabbit antibodies. Multiallergen enzyme-linked immunosorbent assay inhibition and immunoblot methods were conducted using a human serum pool with high levels of IgE to dog allergens.

Results: Dog allergen recoveries ranged from 22% to 134% after short exposures to moderate or extreme temperatures and from 28% to 118% after mixing with fungal or insect extracts and storage for up to 15 months at 2°C to 8°C. Recoveries in dog dander extracts varied up to 2.5-fold with different test methods. Immunoblots revealed partial degradation of dog albumin molecules to discrete fragments that retained antibody-binding activities. In most cases, recoveries improved at elevated glycerin concentrations.

Conclusions: Dog allergens in epithelia and dander extracts exhibited favorable temperature stabilities. Compatibilities with fungal or insect extracts may be compromised or at risk in some combinations. These data support current IT practice parameter recommendations of separating high-protease extracts from other products if possible; they also demonstrate that dog extracts possess allergen stabilities suitable for many IT formulations.

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INTRODUCTION

The development of clinically effective allergen immunotherapy (IT) formulations and protocols remains a fundamental activity in many allergy clinics. Dose recommendations summarized in IT practice parameter guidelines provide suitable targets for standardized allergenic extracts and possible ranges for nonstandardized products.[1] Studies focused on the compatibilities of allergens in different extract mixtures help to identify risky or unstable combinations that can compromise achievement of target doses or consistent delivery of defined allergen levels.[1–4]

Several prominent allergens have been identified in dog epithelia or dog dander source materials. Two allergens in particular, Can f 1 (salivary lipocalin protein) and Can f 3 (dog serum albumin), have been linked to dog allergen sensitivities in 50% and 35% of allergic patients, respectively.[5] Because dog epithelia extracts typically contain high levels of albumin but relatively low levels of Can f 1, and dog dander extracts are usually enriched in Can f 1 but deficient or low in albumin content, many clinics incorporate both types of dog extract on their skin test panels and IT formulations. However, because all commercial dog extracts in the United States are nonstandardized, virtually no data are available addressing the stability of dog allergens or their compatibilities with other allergens in treatment mixtures.

The current study was designed to assess the temperature stabilities of dog allergens with extracts at varying strengths typical of those used in allergy clinics and the compatibilities of dog allergens after mixing with high-protease fungal or insect extracts and storage for up to 15 months at refrigeration temperatures (2°C–8°C).

METHODS

Allergen Extracts

The commercial (licensed) dog extract concentrates used in this study were obtained from Greer Laboratories, Lenoir, North Carolina (dog epithelia, 1:10 wt/vol aqueous and 1:20 wt/vol in 50% glycerin), or Hollister-Stier Laboratories, Spokane, Washington (AP dog hair–dander, 1:50–1:100 wt/vol in 50% glycerin). The following commercial glycerinated extract concentrates were also obtained from Greer Laboratories: *Alternaria alternata* (1:20 wt/vol), *Aspergillus fumigatus* (1:20 wt/vol), *Penicillium chrysogenum (notatum)* (1:20 wt/vol), American cockroach (*Periplaneta americana*, 1:20 wt/vol), German cockroach (*Blatella germanica*, 1:20 wt/vol), and fire ant (*Solenopsis invicta*, 1:20 wt/vol). Extract concentrates were stored at 2°C to 8°C, and all product lots and dilutions were tested within their expiration dates.

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Extract Dilutions and Temperature Incubations

Ten-fold serial dilutions of aqueous dog epithelia extract concentrate were prepared by combining 0.6 mL of extract or previous dilution with 5.4 mL of diluent (human serum albumin [HSA]–saline with 0.4% phenol or 10% glycerin-saline with 0.4% phenol) (Greer Laboratories) to final extract concentrations of 1:100, 1:1,000, and 1:10,000 wt/vol. The extract concentrate and dilutions were incubated for 3 days at temperatures ranging from −18°C to 45°C to examine recoveries to conditions both expected (2°C–8°C, refrigerator; and 20°C–25°C, ambient room temperature) and unexpected but possible during shipping or storage (−18°C, freezer; 34°C, warm shipping environments; and 45°C, hot shipping environments). Glycerinated dog epithelia and dog dander extract concentrates were also diluted 1:10 vol/vol with HSA-saline and heated for 5 minutes at 100°C. Treated samples were stored at 2°C to 8°C and analyzed alongside untreated controls.

Extract Mixtures and Controls

Two-component extract mixtures and single-component controls (2.0-mL volumes) were formulated with each individual extract present at one-tenth of concentrate levels. Final glycerin concentrations of these solutions were adjusted to 10%, 25%, and 50% vol/vol by the addition of nonglycerinated and 50% glycerinated isotonic sodium chloride solution containing 0.4% phenol (Greer Laboratories). Extracts and diluents were combined in 10-mL glass vials and mixed thoroughly. All mixtures and controls were analyzed after storage for up to 15 months at 2°C to 8°C.

Human and Animal Serum Samples

A freeze-dried human serum pool containing specific IgE to dog allergens (Greer Laboratories lot ZE-P2) was used for human IgE enzyme-linked immunosorbent assay (ELISA) and immunoblot analyses. Specific polyclonal rabbit anti–serum samples directed against dog albumin or Can f 1 were developed at Greer Laboratories using purified or partially purified antigens and conventional immunization procedures. Mouse monoclonal anti–Can f 1 was obtained from Indoor Biotechnologies, Charlottesville, Virginia.

Analytical Procedures

Human IgE (hIgE) ELISA inhibition assays were performed as described previously using serum pool ZE-P2 and glycerinated dog epithelia or dog dander extracts as coating and reference reagents.[2] Microtiter plates (Immulon 4; Thermo Electron Corp, Milford, Massachusetts) were coated with 1:500 dilutions of dog extract concentrates in a carbonate buffer (pH 9.6) for 15 to 20 hours at 2°C to 8°C. After washing with phosphate-buffered saline, pH 7.4, containing 0.05% polysorbate 20 (Tween-20), plates were incubated with human serum (1:20 final dilution) containing serial 3-fold dilutions of reference and test samples for 4 to 6 hours at 20°C to 25°C. Bound IgE was then detected by successive incubations with biotinylated anti–human IgE (KPL, Gaithersburg, Maryland), avidin-alkaline phosphatase conjugate (Zymed, San Francisco, California), and *p*-nitrophenyl phosphate chromogenic substrate (Amresco, Solon, Ohio). Absorbance values were determined at 405 nm in a microplate reader (Molecular Devices, Sunnyvale, California). The parallelism and validity of test and reference curves were confirmed by statistical analyses (paired *t* tests), and the relative IgE-binding potencies of test samples were determined using a parallel line bioassay algorithm.

Can f 1–specific double-bind (sandwich) ELISA analyses were conducted using mouse anti–Can f 1 capture antibody, reference antigen, and rabbit anti–Can f 1 probe antibody preparations obtained from Indoor Biotechnologies, and conditions comparable to those recommended by this manufacturer (Nunc Maxi-Sorp microplates; Nalge Nunc International, Rochester, New York). Dog albumin ELISA procedures used Immulon 2 plates (Thermo Electron Corp), a 1:5,000 dilution of rabbit anti–dog albumin in carbonate buffer for coating, serial dilutions of purified dog albumin (Sigma, St Louis, Missouri) as reference, biotinylated rabbit anti–dog albumin, avidin-alkaline phosphatase conjugate, and *p*-nitrophenyl phosphate substrate. Standard curves for Can f 1 and dog albumin ELISA were constructed using absorbance changes over specified periods (reaction rates) and log concentrations of reference antigens. Linear regression analyses identified the reference range exhibiting the highest correlation coefficient, with mean test sample values calculated using all absorbances falling within those of the selected references.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis was performed with 12% polyacrylamide gels using a slab cell system (Mini-Protean II; Bio-Rad, Hercules, California) under nonreducing conditions.[2,6] Test samples and controls were diluted in Laemmli sample buffer (Bio-Rad), boiled for 3 minutes at 100°C, and loaded onto the gels (10 μL per lane). Samples and low-range, 14- to 97-kDa molecular weight standards (1:200 vol/vol dilution in sample buffer) (Bio-Rad) were electrophoresed at a constant voltage (200 V) for 35 to 45 minutes and then transferred by bidirectional passive diffusion to Immobilon-P^{SQ} polyvinylidene difluoride membranes (Millipore, Bedford, Massachusetts) at 20°C to 25°C.[2,7] After blocking, standard lanes were removed and stained with colloidal gold (Bio-Rad). Blot membranes containing test samples were incubated with human serum (1:40 dilution), rabbit anti–Can f 1 (1:100,000 dilution), or rabbit anti–dog albumin (1:500,000 dilution) for 15 to 24 hours at 20°C to 25°C; after 3 washes with Tris-buffered saline–polysorbate (Tween), these membranes were probed with goat anti–human IgE–alkaline phosphatase or goat–anti–rabbit IgG–alkaline phosphatase (Sigma) for 6 hours at 20°C to 25°C. IgE-binding proteins were visualized using the precipitating chromogenic substrate bromochloroindoxyl phosphate–nitroblue tetrazolium (Amresco).

RESULTS

Temperature Stabilities

Dog epithelia and dog dander extract concentrates displayed very high recoveries of allergenic and antigenic activities

after exposures for 3 days to temperatures ranging from −18°C (0°F, freezer) to 45°C (113°F), as illustrated in Table 1. The slightly higher recoveries of Can f 1 activity in dog dander extract may be related to either the higher levels of Can f 1 in this product relative to dog epithelia extract or the presence of 50% glycerin in the dog dander extract concentrate. Dilutions of dog epithelia extract in 10% glycerin-saline or HSA-saline also retained considerable antigenic activities after exposures to the same temperatures (Table 2). For dog epithelia extracts at 1:100 wt/vol, Can f 1 recoveries were consistently higher in the 10% glycerin dilutions, whereas dog albumin recoveries were higher with HSA-saline under all conditions examined.

Immunoblots of dog epithelia extract concentrates and 1:100 wt/vol dilutions in HSA revealed recoveries of IgE- and IgG-binding activities consistent with those produced by ELISA (Fig 1). Human serum IgE molecules were directed primarily against dog albumin, the major component (allergenic or nonallergenic) in dog epithelia extracts. Rabbit anti–dog albumin recognized the exact same extract components as human serum in these blots. Can f 1 bands (25 kDa) were not detected owing to the relatively low concentrations of this allergen (approximately 1 μg/mL) in dog epithelia extract concentrates. Dog epithelia extracts subjected to freezing and thawing also produced no apparent changes in immunoblot reactions with the human and rabbit serum samples (data not shown). Dog allergen recoveries after boiling (5 minutes at 100°C) ranged from 22% to 52% for dog epithelia extracts. For hIgE ELISA inhibition, 1:20 wt/vol, the value was 34%; and 1:200 wt/vol, the value was 39%. For dog albumin ELISA, 1:20 wt/vol, the value was 22%; and 1:200 wt/vol, the value was 52%. These values ranged from 39% to 102% for dog dander extracts. For hIgE ELISA inhibition, 1:100 wt/vol, the value was 64%; and 1:1,000 wt/vol, the value was 53%. For dog albumin ELISA, 1:100 wt/vol, the value was 39%; and 1:1,000 wt/vol, the value was 60%. For Can f 1 ELISA, 1:100 wt/vol, the value was 74%; and 1:1,000 wt/vol, the value was 102%. Dilution (10-fold) of dog extract concentrates in HSA-saline produced considerable improvement in the stability of antigenic structures (Can f 1, albumin) but did not protect IgE-binding epitopes on these or other dog proteins from heat inactivation.

Table 1. Temperature Stabilities of 1:10 wt/vol Aqueous Dog Epithelia and 1:50 wt/vol Glycerinated Dog Dander Extracts

Storage condition	% of 2°C–8°C or 0 freeze-thaw control values			
	Dog epithelia			Dog dander (Can f 1 ELISA)
	hIgE ELISA inhibition	Can f 1 ELISA	Dog albumin ELISA	
3 d at 21°C	90	97	81	ND
3 d at 34°C	ND	119	94	ND
3 d at 45°C	89	94	79	120
Freeze-thaw				
Once	90	84	82	ND
Twice	ND	94	69	ND

Abbreviations: ELISA, enzyme-linked immunosorbent assay; hIgE, human IgE; ND, not determined.

Table 2. Temperature Stabilities of 1:100 wt/vol and 1:1,000 wt/vol Dog Epithelia Extract Dilutions in 10% Glycerol-Saline or HSA-Saline Diluent

Storage condition	% of 2°C–8°C or 0 freeze-thaw control values at each strength					
	1:100 wt/vol				1:1,000 wt/vol (dog albumin ELISA)	
	Can f 1 ELISA		Dog albumin ELISA			
	10%G	HSA	10%G	HSA	10%G	HSA
3 d at 21°C	90	78	60	134	67	91
3 d at 34°C	122	78	64	127	105	102
3 d at 45°C	111	76	71	79	84	78
Freeze-thaw						
Once	109	99	58	91	82	85
Twice	ND	85	ND	85	ND	56

Abbreviations: ELISA, enzyme-linked immunosorbent assay; 10%G, 10% glycerin; HSA, human serum albumin; ND, not determined.

Extract Compatibilities

Dog epithelia extracts mixed with protease-rich fungal extracts (*Alternaria*, *Aspergillus*, or *Penicillium*) or whole-body insect extracts (American cockroach, German cockroach, or fire ant) retained 67% to 113% of their IgE-binding potencies after storage for 13 months at 2°C to 8°C, even at glycerin concentrations as low as 10% (Table 3). On immunoblots, dog albumin bands (66 kDa) were partially degraded to multiple, discrete, lower-molecular-weight fragments after mixing with fungal extracts and, to a lesser degree, insect extracts (Fig 2 and Fig 3). These fragments were recognized by both human IgE and rabbit anti–dog albumin IgG antibodies, demonstrating that at least some epitope structures remained intact after the observed physical changes.

Dog dander extracts mixed with fungal or insect extracts retained 28% to 118% of allergen activities after storage for 12 to 15 months at 2°C to 8°C (Table 3). Can f 1 reactivities in several dog dander extract mixtures varied noticeably from those determined by human IgE ELISA inhibition, with the largest discrepancies observed after mixing with *Aspergillus* or *Penicillium* (2.5-fold higher Can f 1 recoveries) and American cockroach (2.3-fold higher IgE-binding recoveries). Immunoblots of these mixtures and corresponding controls revealed reductions in both Can f 1 and albumin reactivities with *Aspergillus* and *Penicillium* at 10% glycerin (Fig 4). Dog albumin recoveries were relatively high after mixing with insect extracts, with minor changes to Can f 1 observed (Fig 5). For most extract mixtures, higher activities were recovered with increasing glycerin concentrations, similar to results observed previously with mixtures of standardized extracts and high-protease whole-body mold or insect products.[2]

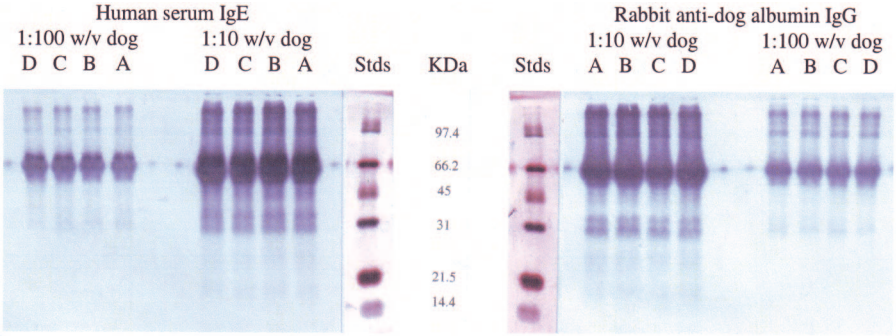

Figure 1. IgE and IgG immunoblot profiles of 1:10 to 1:100 wt/vol dog epithelia extracts and human serum albumin (HSA) saline dilutions after probing with human serum (IgE) or rabbit anti–dog albumin (IgG) antibodies. Lanes 1 through 4 show 1:10 wt/vol dog epithelia extracts, and lanes 5 through 8 show 1:100 wt/vol dog epithelia extracts in HSA-saline diluent. Samples were analyzed after storage for 3 days at 2°C to 8°C (lane A), 21°C (lane B), 34°C (lane C), or 45°C (lane D). Molecular weight standards (Stds) and corresponding kilodalton (KDa) values for immunoblots are illustrated.

Table 3. Recoveries of IgE-Binding Potencies and Specific Allergen Activities of Dog Epithelia and Dog Dander Extracts After Mixing With Fungal or Insect Extracts

Fungal or insect extract added	% Glycerin	% of dog extract control values after storage for 12–15 mo at 2°C–8°C		
		Dog epithelia (hIgE ELISA inhibition)	Dog dander	
			hIgE ELISA inhibition	Can f 1 ELISA
Alternaria alternata	10	94	52	79
	25	99	61	89
	50	107	102	84
Aspergillus fumigatus	10	72	28	69
	25	75	45	90
	50	113	97	98
Penicillium notatum	10	67	40	71
	25	75	46	113
	50	92	118	111
American cockroach	10	82	81	35
	25	87	96	62
	50	80	97	75
German cockroach	10	78	81	59
	25	90	95	88
	50	79	94	94
Fire ant invicta	10	78	74	64
	25	94	103	97
	50	100	112	72

Abbreviations: ELISA, enzyme-linked immunosorbent assay; hIgE, human IgE.

DISCUSSION

IgE-mediated hypersensitivity reactions to proteins derived from dog hair, dander, and epithelia have been described for many years.[8,9] At least 11 and as many as 20 different dog allergens may be responsible for these reactions.[5,10–12] Three prominent allergens (Can f 1, Can f 2, and Can f 3 [albumin]) have been isolated and characterized in terms of their structural and allergenic properties, and recombinant forms of these proteins have also been produced.[13–16] Despite their ubiquitous presence on allergy skin test panels and their frequent inclusion in IT mixtures, dog allergens have only been assessed for stability in one investigation involving short exposures (up to 1 hour) to dry heat (up to 140°C); to our knowledge, no studies have been reported regarding their compatibilities with other, specifically protease-rich, extracts.[17] The formulation of IT mixtures containing dog or other extracts presents a formidable challenge to clinicians who want to balance optimal extract doses with minimal numbers of injections, and it is not surprising to find extract combinations with uncertain allergen stabilities, such as those investigated in this study, being prepared and administered on a regular basis in many allergy clinics.

In the present study, Can f 1 and albumin were found to be the major IgE-binding proteins on immunoblots and exhibited near-complete retention of their allergenic and antigenic activities after exposures to moderate temperatures (20°C–40°C [68°F–104°F]) for up to 3 days. These data provide support for the integrity of dog extracts maintained for short periods at ambient temperatures during routine clinical use or subjected to inadvertent temperature increases during shipping. Brief exposure to a higher temperature (100°C), however, was sufficient to alter the immunochemical properties of dog allergens, as observed in 2-site (sandwich) ELISA and ELISA inhibition analyses. Extracts subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotting are boiled (3 minutes, 100°C) in a denaturing buffer that destroys most (if not all) of the conformational epitopes on dog allergen protein structures. The remaining linear (sequential) binding sites for human IgE (34%–39% for dog epithelia and 53%–64% for dog dander from the present study), rabbit anti–dog albumin IgG (22%–52% for dog epithelia and 39%–60% for dog dander), and

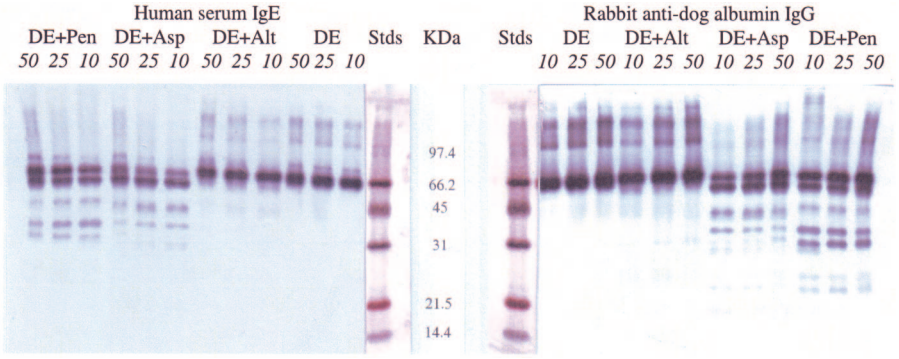

Figure 2. IgE and IgG immunoblot profiles of dog epithelia extracts after mixing with fungal extracts and storage for 13 months at 2°C to 8°C. Blots were probed with human serum (IgE) (left) or rabbit anti–dog albumin (IgG) (right) antibodies. Dog epithelia extracts were analyzed alone (DE) or after mixing with *Alternaria* (Alt), *Aspergillus* (Asp), or *Penicillium* (Pen) extracts. Molecular weight standards (Stds) and corresponding kilodalton (KDa) values for immunoblots are illustrated. Final glycerin concentrations (percentages) in all samples are shown in italics.

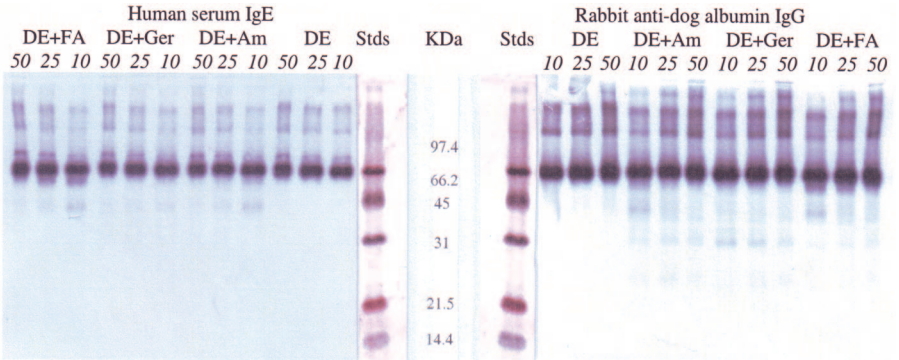

Figure 3. IgE and IgG immunoblot profiles of dog epithelia extracts after mixing with whole-body insect extracts and storage for 13 months at 2°C to 8°C. Blots were probed with human serum (IgE) (left) or rabbit anti–dog albumin (IgG) (right) antibodies. Dog epithelia extracts were analyzed alone (DE) or after mixing with American cockroach (Am), German cockroach (Ger), or fire ant (FA) extracts. Molecular weight standards (Stds) and corresponding kilodalton (KDa) values for immunoblots are illustrated. Final glycerin concentrations (percentages) in all samples are shown in italics.

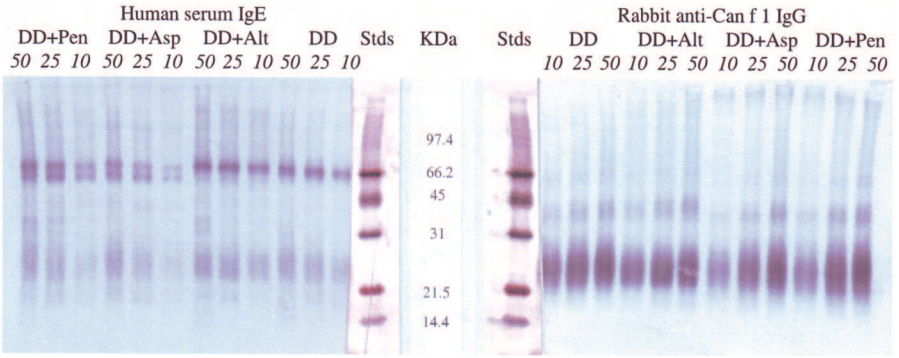

Figure 4. IgE and IgG immunoblot profiles of dog dander extracts after mixing with fungal extracts and storage for 13 months at 2°C to 8°C. Blots were probed with human serum (IgE) (left) or rabbit anti–Can f 1 (IgG) (right) antibodies. Dog dander extracts were analyzed alone (DD) or after mixing with *Alternaria* (Alt), *Aspergillus* (Asp), or *Penicillium* (Pen) extracts. Molecular weight standards (Stds) and corresponding kilodalton (KDa) values for immunoblots are illustrated. Final glycerin concentrations (percentages) in all samples are shown in italics.

mouse or rabbit anti–Can f 1 IgG antibodies (74%–102% for dog dander) represent those visualized on immunoblots. The ELISA procedures involve nondenaturing conditions with unheated or untreated extracts, facilitating antibody interactions with both linear and 3-dimensional structures on dog allergens.

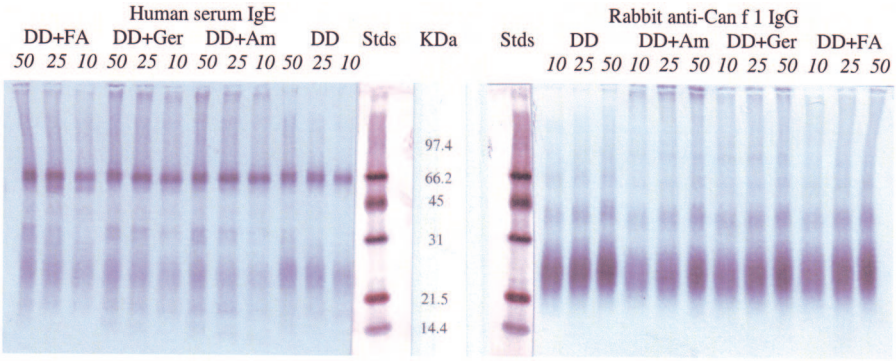

Figure 5. IgE and IgG immunoblot profiles of dog dander extracts after mixing with whole-body insect extracts and storage for 13 months at 2°C to 8°C. Blots were probed with human serum (IgE) (left) or rabbit anti–Can f 1 (IgG) (right) antibodies. Dog dander extracts were analyzed alone (DD) or after mixing with American cockroach (Am), German cockroach (Ger), or fire ant (FA) extracts. Molecular weight standards (Stds) and corresponding kilodalton (KDa) values for immunoblots are illustrated. Final glycerin concentrations (percentages) in all samples are shown in italics.

Mixing dog extracts with several fungal or insect extracts also resulted in reduced dog allergen activities or physical changes in allergen size that could affect the allergenicity or immunogenicity of these molecules in some patients. IgE binding to dog allergens present in dog dander extract (primarily Can f 1) was compromised to a greater degree compared with dog epithelia products (mostly albumin), suggesting that differences in proteolytic enzyme susceptibilities may exist between Can f 1 and albumin. In several cases (*Aspergillus* and *Penicillium*), Can f 1 ELISA activities were considerably higher than the IgE ELISA inhibition reactivities of the same samples, whereas the converse relationship was observed with dog extracts mixed with American cockroach. Thus, although some combinations appeared to retain fairly high levels of allergenic and antigenic activities, mixing dog extracts with fungal or insect products presents a potential risk for degradation or alteration of dog allergens, particularly at low (10%) glycerin concentrations.

An earlier study of dog allergen stability used Can f 1 ELISA reagents produced in different laboratories from those used in the present study.[17–19] To our knowledge, no data are available comparing the specificities of the anti–Can f 1 capture antibodies or the dose-response characteristics of the 2 assays and, thus, the potential exists for these differences to affect the allergen stabilities in dust samples enriched in Can f 1 (earlier study) or those reported herein with aqueous or glycerinated dog extracts. Nevertheless, the relatively high heat tolerance of dog allergens is indicated in both studies.

There are several limitations to the present study. A single dog-positive human serum pool prepared from 4 donors was used for these studies. Individual serum samples may display somewhat different dog allergen specificities and overall allergen recoveries. The extracts used for these investigations included multiple production lots from the 2 allergen manufacturers, but no products from other manufacturers. Extract mixtures containing between 5 and 10 individual products are often formulated for IT in allergy clinics but were excluded here to minimize possible interferences or ambiguities in sample testing. Other allergen combinations (dog extracts with low-protease pollen, cat, or dust mite extracts) should also be investigated. In vivo assessments of extracts and extract mixtures, in combination with these (and other) immunochemical methods, are also needed to support the clinical relevance of the study results.

Observations of variable recoveries from the same test samples using different analytical methods are not unusual or unique to dog allergens.[20–22] Quantitative differences in immunochemical activity have been observed comparing different methods using the same reagents and similar methods (such as ELISA) comparing human IgE– and animal IgG–binding specificities or interactions. The inclusion of a variety of bioanalytical methods in measurements of allergen recoveries in the present study provides additional information on extract properties and the specific changes in allergen composition caused by temperature exposures or combinations with other extracts.

Nonstandardized dog extracts manufactured in the United States are prepared from either dog epithelia (high albumin content and low Can f 1 content) or dog dander (high Can f 1 content and low albumin content) source materials. Ideally, the composition of dog extracts would include moderate (50–100 μg/mL) levels of both Can f 1 and albumin, as well as other minor allergens that may be important for some dog-allergic patients. Prototype hybrid dog epithelia–dog dander extracts have been prepared in this laboratory, and mixtures of commercial dog epithelia and dog dander extracts have been formulated in allergy clinics and incorporated into regular testing and treatment regimens. The standardization of a hybrid dog extract requires establishment of a reference antigen standard that is available for quantitative intradermal skin testing to determine bioequivalent allergy unit activities in an allergic patient population.[23] Subsequent laboratory tests can then be performed using specific allergen (Can f 1, albumin) ELISA, human IgE ELISA inhibition, or other assays, on production lots of these extracts.

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