

This article was downloaded by:[Advanced Institute of Sanita]
On: 27 June 2008
Access Details: [subscription number 790389265]
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954
Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Toxicology and Environmental Health, Part A Current Issues

Publication details, including instructions for authors and subscription information:
<http://www.informaworld.com/smpp/title~content=t713667303>

Environmental Exposure to Allergens of Different Dog Breeds and Relevance in Allergological Diagnostics

Astrid R. R. Heutelbeck ^a, Thomas Schulz ^b, Karl-Christian Bergmann ^c, Ernst Hallier ^d

^a Department of Occupational Health, Georg-August-University, Göttingen

^b Federal Institute for Risk Assessment, Berlin

^c Department of Dermatology and Allergy, Allergie-Centrum-Charité/ECARF, Berlin

^d Department of Occupational Health, Georg-August-University, Göttingen, Germany

Online Publication Date: 01 January 2008

To cite this Article: Heutelbeck, Astrid R. R., Schulz, Thomas, Bergmann, Karl-Christian and Hallier, Ernst (2008) 'Environmental Exposure to Allergens of Different Dog Breeds and Relevance in Allergological Diagnostics', Journal of Toxicology and Environmental Health, Part A, 71:11, 751 — 758

To link to this article: DOI: 10.1080/15287390801985513

URL: <http://dx.doi.org/10.1080/15287390801985513>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article maybe used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Environmental Exposure to Allergens of Different Dog Breeds and Relevance in Allergological Diagnostics

Astrid R. R. Heutelbeck¹, Thomas Schulz², Karl-Christian Bergmann³,
 and Ernst Hallier⁴

¹Department of Occupational Health, Georg-August-University, Göttingen, ²Federal Institute for Risk Assessment, Berlin, ³Department of Dermatology and Allergy, Allergie-Centrum-Charité/ECARF, Berlin, and ⁴Department of Occupational Health, Georg-August-University, Göttingen, Germany

In our environment, dogs are a relevant source of allergens, but diagnosing dog-related allergies may present difficulties, as in diagnostic tests with commercial dog allergens, some patients show only slight positive or negative results, even though they suffer from dog-related symptoms. Occasionally, allergy tests with extracts of dog hair belonging to patients' dogs or from dogs of the same breed were found to yield more reliable results, possibly due to breed-specific allergen components. The purpose of this study was to determine breed-specific differences or possibly hypo- or hyperallergenic dog breeds. The dog allergen content and protein patterns of different commercial and self-prepared dog allergen extracts were compared. Protein extracts were separated using sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stained with silver. The major allergen Can f 1 was quantified using the commercially available enzyme-linked immunosorbent assay (ELISA) technique. The majority of the bands in the self-prepared extracts of different breeds had a molecular mass lower than 30 kD. Notably, the self-prepared extracts of hair of common breeds showed distinct protein bands with a molecular mass lower than 14 kD, which the commercial extracts did not. With regard to Can f 1 content, a marked variability occurred. Factors related to individual dogs seem to influence the allergenicity more than breed or gender. This is the first report to describe allergens with low molecular mass that are absent in extracts of commercial test kits. Consequently, skin tests with self-prepared dog allergen extracts need to be performed in case of inconsistent test results with commercial extracts.

Dogs are a relevant cause of type I allergies and dog allergens are ubiquitous in that they are found in homes with or without dogs, classrooms, day nurseries, and other public

places (Dornelades et al., 1995; Munir et al., 1993). Up to 29 potentially allergenic proteins were identified in dog urine, epidermal scales, serum, and saliva (Blands et al., 1977; Brandt & Yman, 1980; Uhlin et al., 1984; Wüthrich et al., 1995) as well as in particularly high concentrations in hair. Proteins of a molecular mass of 25 kD with an additional component at 18 kD were designated Can f 1 (De Groot et al., 1991; Schou et al., 1991). Another protein fraction with established allergenic potency and a molecular mass between 65 kD and 69 kD was also identified (Uhlin et al., 1984; Yman et al., 1973).

Commercial dog allergen extracts for diagnostic and therapeutic purposes are usually prepared by collecting allergens from nonstandardized sources and are available from different manufacturers. These allergens often show considerable qualitative and quantitative differences, which naturally also affect the results of skin tests, provocation tests, and hyposensitization therapies. At present, no method for standardization of allergens is generally applicable, although an international standard was proposed to ensure valid and comparable diagnostic test results (Larsen et al., 1988). Commercial extracts usually contain a relatively small variety of major allergens to simplify the standardization process.

The prevalence rate of dog allergies within the general adult population amounted to up to 16% (Linneberg et al., 2003; Plaschke et al., 1996; Wüthrich et al., 1995). For asthmatic children, even higher sensitization rates up to 34% were reported (Nelson et al., 1999; Rönmark et al., 2003). Occasionally, however, allergy tests with commercial dog allergen extracts show only slight positive or even negative results even though the tested patients display dog-related clinical symptoms (Wortmann, 1984; Fuchs et al., 1981). Positive reactions with hair from dogs belonging to the patients were reported, without a corresponding result using commercial extracts (Wüthrich et al., 1985; Wortmann, 1984). It would appear that the composition of the dog allergen extracts might play a role in the observed results and that breed-specific

We are grateful for all the support that we received in the course of our study, and we thank, in particular, Petra Tucholla and Thomas Ahrens for laboratorial and Robert Metzner for editorial support.

Professor Karl-Christian Bergmann, MD, was formerly at Allergy and Asthma Clinic, Bad Lippspringe, Germany.

Address correspondence to Astrid R. R. Heutelbeck, Department of Occupational Health, Georg-August-University, Waldweg 37, 37073 Göttingen, Germany. E-mail: aheutel@gwdg.de

allergen components may be responsible (Hooker, 1944). Following this assumption, some dog breeders recommended that owning different dog breeds as "hypoallergenic" might resolve allergic responsiveness, yet there is no supporting evidence for this.

Immunoglobulin (Ig) E-mediated reactions may occur as a response to one or more allergens, but frequently the majority of allergic individuals respond to one "major" allergen while a smaller number of subjects may react to several "minor" allergens. An individual's response to allergens and related sensitization spectrum depends upon the chemical nature of the allergens as well as on the frequency and intensity of contact. The aim of the present study was to assess exposure to multiracial dog allergens in our environment by investigating the respective protein patterns and Can f 1 levels in hair of a wide range of relevant dog breeds. Special attention was paid to the hypothesis that possible factors related to distinct dog breeds are relevant for allergenicity to dogs but are not sufficiently reflected in allergological diagnostic tests.

MATERIAL AND METHODS

Self-Prepared Dog Allergen Extracts

The hair of pure-bred adult dogs was obtained from different owners and breeders throughout Germany. Dogs selected for this study were all healthy to avoid a possible influence of pathology on Can f 1 production. The hair was cut close to the skin without visible contamination. It was confirmed by owners and breeders that dogs had not been washed for 1 wk prior to sampling as recommended (Blands et al., 1977; DeGroot et al., 1991; Schou et al., 1991; Ford et al., 1989). Dog hairs of 36 different breeds were used, including samples of 14 most common dog breeds in Germany (VDH 2005), namely, German shepherd, three different types of dachshund, German wirehaired pointer, Labrador retriever, golden retriever, two different types of poodle, boxer, English cocker spaniel, Rottweiler, giant schnauzer, West Highland white terrier, collie, Airedale terrier, and Bernese mountain. In addition, samples of less common breeds were included, such as Hovawart, miniature Schnauzer, Yorkshire terrier, Chihuahua, Dalmatian, beagle, German spaniel, Briard (Berger de Brie), Irish wolfhound, Siberian husky, Old English sheepdog (bobtail), Shih Tzu, Afghan hound, Bouvier des Flandres (Flanders cattle dog), Shiba Inu, Pekinese, greyhound, American Staffordshire terrier, and Neapolitan mastiff (Mastino Napoletano). The dog breeds were classified according to the Fédération Cynologique Internationale (FCI).

About 0.3 g of hair from each individual dog was extracted for different time periods (2–48 h) at 6°C in 2 ml of a 0.1 M NH_4HCO_3 solution. An incubation period of 24 h was found to yield optimal results in protein content and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) separation (data not shown). The extracts were lyophilized and

reconstituted in NH_4HCO_3 . It was verified that the lyophilized extracts did not show any differences with respect to total protein content or SDS-PAGE separation compared to the unlyophilized extracts (data not shown).

Commercial Dog Allergen Extracts

Raw material from four different manufacturers of skin test extracts (Allergopharma, Reinbek bei Hamburg, Germany; ALK-Scherax, Hamburg, Germany; Bencard, Munich, Germany; and HAL, Düsseldorf, Germany, here after referred to as A, B, C, and D, respectively) was used. Manufacturer A separately provided raw material from two different sources, namely, extracts made of hair and skin scrapes (A_{hs}) from German shepherd, poodle, boxer, collie, and dachshund, as well as extracts made of dander (A_{d}) from German shepherd and poodle. Manufacturer B stated that the raw material was obtained from different dog breeds without further specification. Manufacturer C used hair and epithelium from German shepherd, golden retriever, English cocker spaniel, Scottish terrier, poodle, terrier, collie, Airedale terrier, Labrador, Irish setter, Bernese mountain, German shorthaired pointer, Rottweiler, German wirehaired pointer, fox terrier, and Doberman in its raw material. Manufacturer D extracted its raw material from hair and dandruff brushed from the fur of poodles (20%) and longhaired dogs. After reconstitution of the lyophilized raw material in 0.1 M NH_4HCO_3 solution, the total protein content was about 4 mg/ml.

Protein Determination

Protein content was determined using the bicinchonic acid procedure as described by Pierce Chemicals, USA. The results were verified using several dilutions of each sample.

Determination of the Protein Patterns (SDS-PAGE)

Proteins were separated using SDS-PAGE. A 15% separating gel was used to achieve optimum separation of proteins in the relative molecular mass between 6 and 60 kD. Molecular weights were estimated by comparison to commercial molecular mass standard mixtures ("SDS Low Range" from Bio-Rad, Munich, Germany; "Multi Mark" from Invitrogen, Karlsruhe, Germany). Equal amounts of proteins concentrated at 0.2 mg/ml (silver staining) or 2 mg/ml (immunoblotting) were applied for PAGE, which was conducted at a constant voltage (150 V) for 90–100 min. The two different marker protein preparations were run alongside the extracts. For the investigation of the protein patterns, the gels were analyzed using the Silver Quest kit (Invitrogen, Karlsruhe, Germany). The molecular masses of the corresponding allergens were estimated relative to the standard marker proteins. Each sample was investigated at least twice in independent experiments.

Patient Sera

The sera of three persons (one male, two female, age between 46 and 61 yr, mean 56 yr) with dog-related symptoms, but negative test results with commercial dog allergen extract were used. These patients were examined in the Allergy and Asthma Clinic under suspicion of an allergic airways disease with rhinitis and asthmatic symptoms. One of them still had dogs at home (terrier); two of them were former dog owners (poodle, German shepherd). In tests with different commercial dog allergen extracts, neither serum antibodies nor specific skin test reactivity was detected for any of the three.

Detection of Allergens (Immunoblotting)

The detection of the allergenic proteins in the extracts was performed by immunoblotting. After separation by SDS-PAGE on a 15% gel, proteins were transferred onto polyvinylidene difluoride (PVDF) membranes in a semi-dry blot apparatus. Membranes were incubated overnight in Roti Block solution (Roth, Karlsruhe, Germany) to block nonspecific binding sites and finally incubated with two serum dilutions (1:5 and 1:20) for 1 h at room temperature. After washing 5 times with TBS containing 0.1% Tween, anti-human-IgE monoclonal antibodies diluted 1:1000 coupled with alkaline phosphatase (Sigma, Deisenhofen, Germany) were added for 1 h at room temperature. After washing 5 times with TBS containing 0.1% Tween, the detection of alkaline phosphatase was performed using the NBT (*p*-nitro blue tetrazolium chloride)/BCIP (5-bromo-4-chloro-3-indoyl phosphate *p*-toluidine salt) system (Bio-Rad, Munich, Germany) according to the recommendations of the manufacturer.

Can f 1 Quantification

The major allergen Can f 1 was assayed for a few dog breeds which were represented by two or more individual dogs. A commercially available enzyme-linked immunosorbent assay (ELISA) was used, which is based on the detection of the major allergen Can f 1 (Indoor Biotechnologies, UK) (Schou et al., 1992). Microplates were coated by the monoclonal antibody 6E9F6, anti Can f 1. Nonspecific sites were saturated by PBST-BSA (bovine serum albumin) (Serva, Germany). The Can f 1 standard ranged from 1 to 500 ng/ml. A polyclonal rabbit serum against dog extracts was used as detection antibody. Samples were incubated with a peroxidase-conjugated goat anti-rabbit IgG (Jackson Immuno Research, USA). An ABTS/H₂O₂ substrate solution (2,2-azino-di[3-ethylbenzthiazolinesulfonate diammonium salt) (Roche Molecular Biochemicals, Germany) was added to reveal the reaction. The optical densities were read with a 405-nm filter after 10 min. Results are presented as micrograms per gram hair.

RESULTS

Investigation of the Protein Patterns in Dog Allergen Extracts

Self-Prepared Extracts

A number of dog allergen extracts were investigated using SDS-PAGE (Figures 1 and 2). Each lane represents an extract of hair of one individual dog. The raw material of various male and female individual dogs of the same breed was investigated when hair was available. In the self-prepared extracts, clear bands were distinguished at molecular mass around 6, 8, 14, 18/19, 20, 25, 30, 55, 66, 69, and above 97 kD. In addition, several small bands were found at molecular mass around 16, below 14, and between 30 and 43 kD. Most of the bands appeared at molecular mass below 30 kD (Table 1). Proteins of

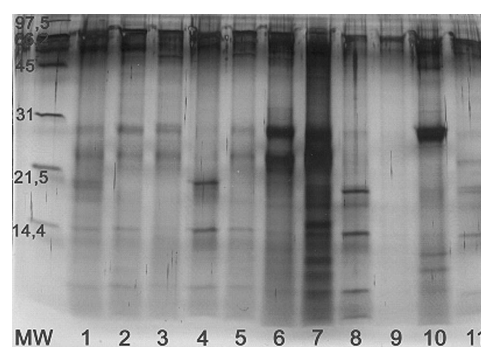


FIG. 1. SDS-PAGE analysis of allergen extracts from eleven individual dogs. Proteins were separated on a 15% gel. Molecular weight markers are shown (MW). Lanes were loaded with 3 μ g of total protein. The following samples were used: lanes 1 and 2 female Yorkshire Terrier, lanes 3 to 6 male Yorkshire Terrier, lane 7 female Poodle and lanes 8 to 11 male Poodle.

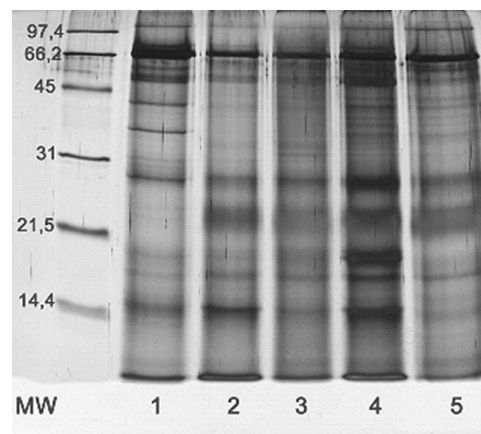


FIG. 2. SDS-PAGE analysis of five commercial dog allergen extracts. Proteins were separated on a 15% gel. Molecular weight markers are shown (MW). Lanes were loaded with 4 μ g of total protein. The following samples were used: lane 1 manufacturer A (hair and skinscape), lane 2 manufacturer A (dander), lane 3 manufacturer B, lane 4 manufacturer C and lane 5 manufacturer D.

TABLE 1
Protein Bands (Determined by SDS-PAGE) in Self-Prepared Dog
Allergen Extracts from Hair of Pure Bred Dogs of Different Breeds

FCI Group	Breeds of Dog	Molecular weight [kDa]																	
		~6	~8	>8	<14	14,5 (15)	~16	~18/19	~20	21,5	~25	~30	31	>31	<45	45	~55	66	>66
1	Bouvier des Flandres / Flanders Cattle Dog (n = 1)			x		x		x	x		x	x					x	x	
1	Briard / Berger de Brie (n = 1)			x		x			x										
1	Collie (n = 2)		x	x		x						x					x	x	
1	German Shepherd Dog (n = 8)		x	x		x					x	x		x			x	x	
1	Old English Sheepdog / Bobtail (n = 2)		x	x		x		x	x		x	x					x	x	
2	Bernese Mountain Dog (n = 2)			x		x						x					x	x	
2	Boxer (n = 3)			x								x					x	x	
2	Giant Schnauzer (n = 1)					x					x	x	x	x	x			x	
2	Hovawart (n = 1)			x		x			x		x	x					x	x	
2	Miniature Schnauzer (n = 1)			x		x		x	x		x						x	x	
2	Neapolitan Mastiff / Mastino Napoletano (n = 1)			x		x						x				x	x	x	
2	Rottweiler (n = 2)			x		x						x							x
3	Airedale Terrier (n = 1)			x		x		x	x			x					x	x	
3	American Staffordshire Terrier (n = 1)		x	x	x	x			x		x	x					x	x	
3	West Highland White Terrier (n = 5)			x	x	x		x	x		x	x		x			x	x	x
3	Yorkshire Terrier (n = 8)		x	x	x	x		x	x		x	x		x			x	x	x
4	Long-haired standard Dachshund (n = 7)			x				x			x	x		x			x	x	x
4	Miniature wire-haired Dachshund (n = 2)					x			x		x	x							
4	Wire-haired Dachshund (n = 5)					x		x			x	x					x	x	
5	Shiba Inu (n = 1)		x	x							x						x	x	
5	Siberian Husky (n = 2)		x	x		x		x			x	x					x	x	x
6	Beagle (n = 1)					x		x			x	x					x	x	
6	Dalmatian (n = 1)					x		x	x		x	x		x			x	x	
7	German Wirehaired Pointer (n = 1)					x		x	x		x	x	x				x	x	
8	English Cocker Spaniel (n = 8)			x	x	x		x	x		x	x		x			x	x	x
8	German Spaniel (n = 3)			x	x	x		x	x		x	x		x		x	x	x	x
8	Golden Retriever (n = 3)				x	x		x	x			x		x			x	x	
8	Labrador Retriever (n = 1)					x		x	x		x	x	x	x			x	x	x
9	Chihuahua (n = 1)																	x	
9	Miniature Poodle (n = 4)			x		x					x						x	x	x
9	Pekingese (n = 1)			x		x			x									x	
9	Poodle (n = 6)			x	x	x		x	x		x	x					x	x	x
9	Shih Tzu (n = 2)			x		x			x		x	x					x	x	x
10	Afghan Hound (n = 6)			x	x	x		x	x		x	x		x					
10	Greyhound (n = 4)			x	x	x		x	x		x	x		x		x	x	x	
10	Irish Wolfhound (n = 3)			x	x	x		x	x		x	x		x		x	x	x	

a molecular mass of 25 kD appeared as a double band with minor and major components corresponding to the major allergen Can f 1. These proteins were detected in the extracts of more than 70% of the investigated dog breeds.

The extracts of every third breed showed one or more weak bands at a molecular mass below 14 kD. Particularly divergent breeds such as terrier, poodle, collie, German shepherd, Old English sheep, Shiba Inu, Siberian husky, English cocker spaniel, German spaniel, Afghan hound, greyhound, Irish wolfhound, and Shih Tzu showed clear protein bands at molecular mass between 6 and 14 kD. No corresponding bands were found in commercial extracts. In the extracts of nearly all breeds, another protein fraction was detected at a molecular mass between 65 and 69 kD, which corresponding to dog albumin. In addition, various small protein fractions at molecular mass between 30 and 43 kD appeared in the extracts of every third breed.

The tests revealed no striking breed- or gender-specific protein bands. Instead, a high variability of the protein bands was seen even among extracts of dogs of the same breed, including long-haired standard dachshund, Yorkshire terrier (Figure 1), poodle (Figure 1), and Afghan hound and Irish wolfhound (data not shown). For some individual dogs such as collie, Briard (Berger de Brie), Bernese mountain, boxer, Neapolitan mastiff (Mastino Napoletano), Rottweiler, Airedale terrier, golden retriever, Chihuahua, and Pekingese, a protein band at 25 kD was detected. Most extracts also showed bands corresponding to dog albumin (data not shown).

Commercial Extracts

Each lane in Figure 2 represents a commercial extract; two extracts from manufacturer A (two kinds of raw material: hair and skin scraping, dander) as well as extracts from manufacturers B, C, and D. In all 5 commercial extracts, distinct protein fractions were observed at about 14, 18/19, 20, 25, 30, 43, 55, 66, 69, and above 97 kD. In addition, most of the commercial extracts showed strong double bands at molecular mass around 6 kD (except extract A_d) and 38 kD (except extracts A_d and B). Several thin bands were also observed at molecular mass around 16, between 6 and 14, and between 31 and 43 kD. Comparing the extract of hair and skin scraping (A_{hs}) with the extract of dander (A_d), it was noteworthy that the latter showed a strong band, the former only a weak band at a molecular mass 25 kD (Can f 1), and additional bands at molecular mass between 31 and 45 kD.

Detection of the Allergen Patterns

In the immunoblot experiments, the four commercial dog allergen extracts from manufacturers A, B, and C were used (the extract of manufacturer D was not included in all experiments due to a lack of raw material), as well as extracts of hair from patients' own dogs. Typical results for extracts of two German

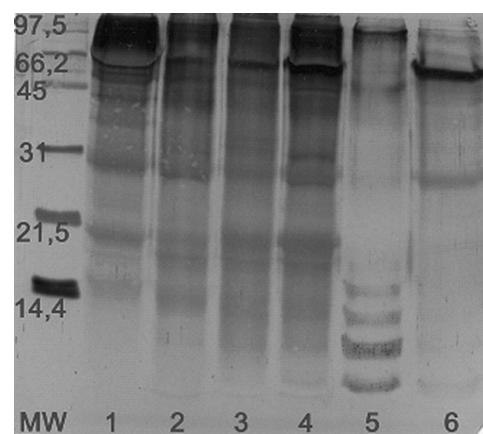


FIG. 3. Immunoblot of allergen extracts from individual dogs and commercial extracts. Proteins were separated by SDS-PAGE (15% acrylamide) and transferred to PVDF membranes. These were developed with human serum. Lanes were loaded with 40 µg of total protein. The following samples were used: lanes 1 manufacturer A (hair and skinscape), lane 2 manufacturer A (dander), lane 3 manufacturer B, lane 4 manufacturer C, lane 5 male German Shepherd Dog and lane 6 female German Shepherd Dog.

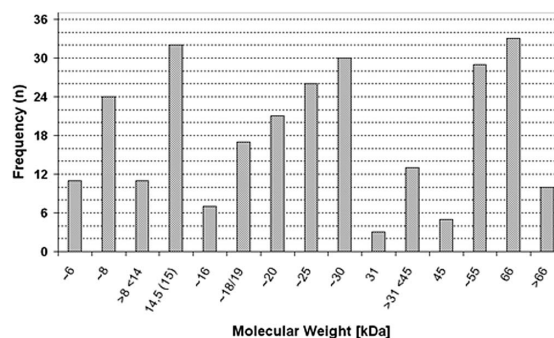


FIG. 4. Frequency (n) of the protein bands (determined by SDS-PAGE) in self-prepared dog allergen extracts of hair of pure bred dogs of 36 different breeds.

shepherds (one male and one female) are shown in Figure 3. Additional immunoblots were performed with two poodle extracts (one male and one female) and with extracts of different terriers (one male and one female West Highland white terrier, one male and one female Yorkshire terrier, and one male Airedale terrier) and were developed with sera of respective dog owners (data not shown).

In general, a high reactivity was found with different serum concentrations using the extracts of the male German shepherd, the female poodle, the male West Highland white terrier, and the male Yorkshire terrier at molecular mass below 20 kD. No corresponding immunoreactions were noted in the commercial extracts. For the female German shepherd, different terrier dogs, and poodle, a certain degree of immunoreactivity was observed at high molecular mass corresponding to dog albumin. With

regard to Can f 1 immunoreactivity, more reactions were noted at molecular mass 18 kD corresponding to the minor components of Can f 1 rather than at molecular mass 25 kD reflecting the major components. Using the extract A_{hs}, immunoreactivity was found at molecular mass between 31 and 45 kD without corresponding reactions in the other commercial extracts. The tests also revealed differences in immunoreaction patterns between male and female dogs of the same breed. Negative controls, performed without serum and with serum of nonsensitized persons showed no reactivity in immunoblotting (data not shown).

Hair Can f 1 Levels According to Breeds and Gender

The analysis was limited to the breeds with more than at least two individuals in each group; the extracts of 32 different dogs were investigated (Table 2). The amount of Can f 1 in the tested hair samples differed more than 10-fold. A large variability in Can f 1 levels in the hair was observed between different breeds, but also between individual dogs of the same breed. Individual dogs showing lower levels of Can f 1 in their hair included German shepherds and Bernese mountain dogs, while the highest Can f 1 levels were found for some Yorkshire terriers, poodles, and miniature poodles. Differences in the Can f 1 levels in hair samples were not related to the breed or gender of the respective dogs.

DISCUSSION

The aim of the present study was to assess exposure to multiracial dog allergens in our environment by investigating the respective protein patterns and hair Can f 1 levels for a wide range of relevant dog breeds. Of particular interest were possible factors related to distinct dog breeds that are relevant to observed allergenicity of dogs but that are not yet sufficiently reflected in allergological diagnostic tests and that might explain the occasional clinical observation that patients with dog-related symptoms react only to certain dog extracts and not other.

The existence of breed-specific sensitizations is well known. As early as 1944, heterogeneous skin test results with different dog breeds were described (Hooker, 1944). Thereafter, several studies dealt with the differences of dog allergen extracts that were manufactured using different *in vivo* and *in vitro* methods. Some of these investigations confirmed qualitative and quantitative differences between dog breeds using quantitative immunoelectrophoresis experiments (Blands et al., 1977; Uhlin et al., 1984). Several other investigations confirmed positive results of varying intensity in comparing extracts of different dog breeds using skin or RAST tests (Fuchs et al., 1981; Lindgren et al., 1988; Moore & Hyde, 1980; Fagerberg & Wide, 1970). By skin testing in a symptomatic patient, the extract of his own St. Bernard dog yielded a negative result in contrast to a highly positive one with an extract from boxer (Wortmann, 1984). Our results are in agreement with these investigations in that there was a large variability of protein patterns between different dog breeds. Further, no clear breed- or gender-specific protein components were revealed.

The protein patterns of our self-prepared extracts from different dog breeds showed bands at molecular mass as previously reported (Schou et al., 1991; Yman et al., 1973; Boutin et al., 1988; Spitzauer et al., 1993), with Can f 1 and dog albumin being the proteins that were most frequently detected. However, it is noteworthy that in our experiments Can f 1-equivalent proteins were not contained in extracts of common breeds such as Airedale terrier, golden retriever, collie, Bernese mountain, boxer, and Rottweiler, which were mainly characterized by proteins bands at other molecular masses. Moreover, and even more important, our experiments revealed not only a wide spectrum of proteins in the self-prepared extracts, but also major differences in the protein patterns between individual dogs of the same breed, including poodle, long-haired standard dachshund, Yorkshire terrier, Afghan hound, and Irish wolfhound. Every individual dog has a distinct protein pattern, which is weakly correlated to the breed, but independent of

TABLE 2
Can f 1 Levels in Self-Prepared Dog Allergen Extracts of Hair of Pure Bred Dogs of Different Breed

Breed	Number (n)	Minimum Can f 1 µg/g hair	Maximum Can f 1 µg/g hair	Geometric mean Can f 1 µg/g hair	Median Can f 1 µg/g hair	Can f 1 µg/mg protein (mean)
German Shepherd	4	11.49	766.90	214.83	40.47	9.47
Bernese Mountain Dog	2	33.64	97.45		65.54	2.51
West Highland White Terrier	4	55.92	371.64	273.47	261.15	10.76
Yorkshire Terrier	5	117.91	1033.49	527.21	534.88	30.72
Wire-haired Dachshund	3	85.02	222.08	149.00	139.90	6.54
English Cocker Spaniel	3	69.25	303.78	170.36	138.05	7.86
Poodle	5	118.80	1189.72	359.23	145.36	15.11
Shih Tzu	2	109.15	710.51		409.83	15.28
Miniature Poodle	4	166.95	1237.40	682.51	662.85	25.53

gender. Observations on individual-specific allergenic components are well established (Wortmann, 1984; Hansen, 1940; Rudolph et al., 1981). Thus the individuality of protein patterns may have been responsible for the observed differences in skin testing results. It therefore seems to be advisable to use the hair of more than one individual dog in allergy tests with self-prepared extracts in order to include all relevant proteins.

With regard to the commercial allergen extracts, our investigations showed, in contrast to quantitative and qualitative differences as well as heterogenic skin test results that were previously described (Larsen et al., 1988; Dreborg, 1993; Vanto et al., 1980; Drouet et al., 1996), only minor differences in the protein patterns. However, commercial dog allergen extracts are a mixture of dog material such as hair and/or dander from various origins. The major allergen Can f 1 appears to be adequately represented only in dander and to a much lesser extent in hair and skin scraping extracts (Martinez et al., 1994). However, the hair and skin scraping showed clear protein bands between 30 and 43 kD, the allergenic relevance of which was shown by Spitzauer et al. (1991), and which were absent in dander extracts. At present, the standardization of commercial allergen extracts is focused on only a small number of important allergens such as Can f 1. Yet in the extracts of individual dogs of distinct breeds such as Bernese mountain, boxer, Neapolitan mastiff (Mastino Napoletano), Rottweiler, Siberian husky, beagle, and Chihuahua, Can f 1 was not identified as a relevant component. Consequently, the allergen pattern of some breeds was possibly not reflected in extracts that were standardized with special regard to the Can f 1 content.

The most striking finding was the demonstration of the allergenic significance of proteins of molecular mass below 14 kD, which was taken into consideration only quite rarely in earlier investigations. Our findings substantiate the quantitative relevance of these proteins, as they were found in more than 70% of our self-prepared extracts. In addition, our immunoblot experiments allowed us to demonstrate for the first time the allergenic relevance of proteins of low molecular mass around 6 and 9 kD for some sensitized patients. These findings are in marked contrast to an investigation that noted these proteins (i.e., proteins with molecular mass below 15 kD) to be nonallergenic (McLean et al., 1980), while other investigations did not even examine proteins of these low molecular mass values.

In addition to the chemical nature of the dog allergens, the intensity of the contact determines sensitization. Can f 1 levels found in air may differ widely in homes with dogs, from 0.3 to 99 ng per m³ (Egmar et al., 1998). These variations may be linked to (1) environmental factors, (2) reservoirs of dog allergens in the homes, or (3) avoidance measures taken. They may also be linked to the characteristics of dogs in the respective home, such as the number of dogs, gender, or breed. The influence of such factors on the production of Can f 1 was evaluated among 288 dogs of different breeds, such as German shepherd, Pyrenean shepherd, poodle, English cocker spaniel, spaniel, griffon, Labrador retriever, and Yorkshire terrier. Data showed

that particular characteristics of certain breeds such as Labrador retriever, the gender and seborrhea seem to influence the levels of Can f 1 on fur (Ramadour et al., 2005). In agreement with these results, dogs of some breeds, including poodle and Yorkshire terrier, were found to have particularly high quantities of Can f 1 in hair. It is well established that dog-specific factors influence the concentration such that Can f 1 quantities in hair differ markedly, up to 70-fold, between individual dogs of the same breed. Furthermore, individual factors seem to be more relevant than breed-specific factors. In contrast to other studies (Ramadour et al., 2005), our results do not confirm the influence of gender-specific factors on hair Can f 1 quantities. Nevertheless, every fifth dog breed did not show relevant protein amounts comparable to Can f 1. Since Can f 1 quantification is the most common method of estimating dog allergen exposure in our environment, there may be a serious underestimation due to the lack of presence of Can f 1 in certain dogs.

In order to improve the accuracy of the results of allergen testing in the future, it is recommended to include proteins of lower molecular mass in the extracts as a consequence of their relevance as allergens as demonstrated by our findings. Overall, individual factors seem to be more important for allergenicity than breed or gender. A hypo- or hyperallergenic dog breed does not exist. In conclusion, there are a few practical consequences. Test results with commercial extracts that are inconsistent with clinical symptoms need to be corroborated by skin tests using extracts of hair belonging dogs of patients. Further immunoblot experiments are necessary to elucidate whether individual allergen extracts sufficiently reflect the individual sensitization patterns compared to commercial allergen extracts. In the light of our results, the proposed international standard for dog hair/dander extracts (Larsen et al., 1988) needs to be reexamined, especially with regard to the estimation of the antigen content.

REFERENCES

- Blands, J., Löwenstein, H., and Weeke, B. 1997. Characterization of extract of dog hair and dandruff from six different dog breeds by quantitative immunoelectrophoresis. Identification of allergens by crossed radioimmunoelectrophoresis (CRIE). *Acta Allergol.* 32:147–169.
- Boutin, Y., Hebert, H., Vrancken, E. R., and Mourad, W. 1988. Allergenicity and cross-reactivity of cat and dog allergenic extracts. *Clin. Allergy* 18:287–293.
- Brandt, R., and Yman, L. 1980. Dog dander allergens: Specificity studies based on the radioallergosorbent technique. *Int. Arch. Allergy Appl. Immunol.* 61:361–370.
- De Groot, H., Goei, K. G. H., van Swieten, P., and Aalberse, R. C. 1991. Affinity purification of a major and a minor allergen from dog extract: serologic activity of affinity-purified Can f I and Can f I-depleted extract. *J. Allergy Clin. Immunol.* 87:1056–1065.
- Dornelades Andrade de, A., Chapin, D., Birnbaum, J., Lanteaume, A., Chapman, M., and Vervloet, D. 1995. Clinical aspects of allergic disease. Indoor allergen levels in day nurseries. *J. Allergy Clin. Immunol.* 95:1158–1163.
- Dreborg, S. 1993. The batch to batch variation of the potency of dog- and cat-allergen-coated lancets. Evaluation by skin prick testing. *Allergy* 48:373–376.
- Drouet, M., Nicolie, B., Coipeau, P., Bonneau, J. C., and Sabbah, A. 1996. Assay of IgE specific to dog epithelia by Pharmacia CAP RAST technique: Comparison of two CAP RAST e2 and e5. *Allerg. Immunol.* 106:109–111.

- Egmar, A. C., Emenius, G., Almqvist, C., and Wickman, M. 1998. Cat and dog allergen in mattresses and textile covered floors of homes which do or not have pets, either in the past or currently. *Pediatr. Allergy Immunol.* 9:31–35.
- Fagerberg, E., and Wide, L. 1970. Diagnosis of hypersensitivity to dog epithelium in patients with asthma bronchiale. *Int. Arch. Allergy Appl. Immunol.* 39:301–309.
- Ford, A. W., Alterman, L., and Kemeny, D. M. 1989. The allergens of dog. I. Identification using cross-immuno-electrophoresis. *Clin. Exp. Allergy* 19:183–190.
- Fuchs, E., Gronemeyer, W., and Bandilla, K. 1981. Reibtest und Tierhaarallergie, zugleich ein klinischer Beitrag zum Problem der "Rassespezifität". *Allergologie* 4:241–248.
- Hansen, K. 1940. Die exogenen Allergene. In *Allergie*, eds. W. Berger and K. Hansen, p. 127. Leipzig: Thieme.
- Hooker, S. B. 1944. Qualitative differences among canine danders. *Ann. Allergy* 2:281.
- Larsen, J. N., Ford, A., Gjesing, B., Levy, D., Petrunov, B., Silvestri, L., and Löwenstein, H. 1988. The collaborative study of the international standard of dog, *Canis domesticus*, hair/dander extract. *J. Allergy Clin. Immunol.* 82:318–330.
- Lindgren, S., Belin, L., Dreborg, S., Einarsson, R., and Pahlman, I. 1988. Breed-specific dog-dandruff allergens. *J. Allergy Clin. Immunol.* 82:196–204.
- Linneberg, A., Nielsen, N. H., Madsen, F., Frolund, L., Dirksen, A., and Jorgensen, T. 2003. Pets in the home and the development of pet allergy in adulthood. The Copenhagen Allergy Study. *Allergy* 58:21–26.
- Martinez, A., Martinez, J., Sanz, M.L., Bartolome, B., and Palacios, R. 1994. Dander is the best epithelial source for dog allergenic extracts preparation. *Allergy* 49:664–667.
- McLean, A. C., Glovsky, M. M., Hoffman, D. R., and Ghekiere, L. M. 1980. Identification of allergens in dog dander extracts. I. Clinical and immunological aspects of allergenic activity. *Ann. Allergy* 45:199–204.
- Moore, B. S., and Hyde, J. S. 1980. Breed-specific dog hypersensitivity in humans. *J. Allergy Clin. Immunol.* 66:198–203.
- Munir, A. R. M., Einarsson, R., Schou, C., and Dreborg, S. K. G. 1993. Allergens in school dust. *J. Allergy Clin. Immunol.* 91:1067–1074.
- Nelson, H. S., Szefer, S. J., Jacobs, J., Huss, K., Shapiro, G., and Sternberg, A. L. 1999. The relationships among environmental allergen sensitization, allergen exposure, pulmonary function, and bronchial hyperresponsiveness in the Childhood Asthma Management Program. *J. Allergy Clin. Immunol.* 104:775–785.
- Plaschke, P., Janson, C., Norrman, E., Bjornsson, E., Lundback, B., Lindholm, N., Rosenhall, L., Jarvholm, B., and Boman, G. 1996. Skin prick tests and specific IgE in adults from three different areas of Sweden. *Allergy* 51:461–472.
- Ramadour, M., Guetat, M., Guetat, J., El Biaz, M., Magnan, A., and Vervloet, D. 2005. Dog factor differences in Can f 1 allergen production. *Allergy* 60:1060–1064.
- Rönmark, E., Perzanowski, M., Platts-Mills, T., and Lundback, B. 2003. Different sensitization profile for asthma, rhinitis, and eczema among 7–8-year-old children: Report from the Obstructive Lung Disease in Northern Sweden studies. *Pediatr. Allergy Immunol.* 14:91–99.
- Rudolph, R., Kunkel, G., Blohm, B., Muckelmann, R., Mast, H., Kirchhof, E., and Sladek, M. 1981. Zur Häufigkeit und klinischen Bedeutung von Allergien gegen Tierepithelien. *Allergologie* 4:230–236.
- Schou, C., Svendsen, U. G., and Löwenstein, H. 1991. Purification and characterization of the major dog allergen Can f I. *Clin. Exp. Allergy* 21:321–328.
- Schou, C., Hansen, G. N., Lintner, T., and Löwenstein, H. 1992. Assay for the major dog allergen, Can f 1: Investigation of house dust samples and commercial dog extracts. *J. Allergy Clin. Immunol.* 88:847–853.
- Spitzauer, S., Rumpold, H., Ebner, C., Schweiger, C., Valenta, R., Gabl, F., Anrather, J., Breitenbach, M., Scheiner, O., and Kraft, D. 1991. Allergen profiles of dog hair and dander, body fluids and tissues as defined by immunoblotting. *Int. Arch. Allergy Appl. Immunol.* 94:346–348.
- Spitzauer, S., Schweiger, C., Anrather, J., Ebner, C., Scheiner, O., Kraft, D., and Rumpold, H. 1993. Characterization of dog allergens by means of immunoblotting. *Int. Arch. Allergy Immunol.* 100:60–67.
- Uhlin, T., Reuterby, J., and Einarsson, R. 1984. Antigenic/allergenic composition of poodle/Alsatian dandruff extract. *Allergy* 39:125–133.
- Vanto, T., Viander, M., and Koivikko, A. 1980. Skin prick test in the diagnosis of dog dander allergy: A comparison of different extracts with clinical history, provocation tests and RAST. *Clin. Allergy* 10:121–132.
- Verband für das Deutsche Hundewesen (VDH). http://www.vdh.de/alles_fuer_medien/welpenstatistik_list.php. 2005.
- Wortmann, F. 1984. Sensibilisierungen gegenüber Haaren und Epithelien verschiedener Tierindividuen (bei fraglicher Rasseidentität)—Bedeutung der Testung mit Material des patienteneigenen Allergenspenders. *Allergologie* 7:69–73.
- Wüthrich, B., Guerin, B., and Hewitt, B. E. 1985. Cross-allergenicity between extracts of hair from different dog breeds and cat fur. *Clin. Allergy* 15:87–93.
- Wüthrich, B., Schindler, C., Leuenberger, P., and Ackermann-Lieblich, U. 1995. Prevalence of atopy and pollinosis in the adult population of Switzerland (SAPALDIA study). Swiss Study on Air Pollution and Lung Diseases in Adults. *Int. Arch. Allergy Immunol.* 106:149–156.
- Yman, L., Brandt, R., and Ponterius, G. 1973. Serum albumin—An important allergen in dog epithelia extracts. *Int. Arch. Allergy* 44:358–368.