

The novel high molecular weight *Dermatophagoides farinae* protein Zen-1 is a major allergen in North American and European mite allergic dogs with atopic dermatitis

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Background – Atopic dogs with hypersensitivity to *Dermatophagoides farinae* (Df) have IgE recognizing high molecular weight (MW) allergens more often than the low MW Der f 1 and 2. A new high MW Df allergen, Zen-1, has been identified recently.

Objectives – To determine the IgE reactivity of American and European Df-hypersensitive dogs to Zen-1, Der f 1 and Der f 2.

Methods – We tested sera from 33 Df-reactive dogs from the USA, 29 from Europe and 15 experimentally sensitized to Df, by ELISA against crude Df, Der f 1, Der f 2 and Zen-1. ELISA inhibition was performed with sera reactive to Zen-1. Intradermal testing (IDT) was also done with the same allergens in 25 other American atopic dogs.

Results – Altogether, IgE seropositivity to Zen-1 was more prevalent (86%) than that to Der f 1 (17%) or Der f 2 (19%). The IgE reactivity to Zen-1 was correlated to that against crude Df; this allergen alone inhibited a high percentage (median: 50%; range: 22–84%) of the binding to the crude mite extract. The seropositivity to low MW allergens was highest in experimentally sensitized dogs. Serum IgE recognition of Der f 1 was low in dogs with AD; that to Der f 2 was significantly lower in American dogs (6%) than in European ones (28%). A high prevalence of positive immediate IDT reactions to Zen-1 confirmed the likely relevance of serological results.

Conclusions and clinical importance – This study establishes Zen-1 as a major allergen in atopic dogs sensitized to Df.

Introduction

Dogs with atopic dermatitis (AD) commonly exhibit high serum IgE against environmental and food allergens.^{1,2} Such allergen-specific IgE are believed to underlie the development of AD skin lesions, at least in most patients; this assertion is supported by the observation that allergic inflammation after epicutaneous challenges is restricted to allergens against which the dog has elevated serum

IgE.³ Among environmental allergens, dogs with AD most commonly mount an IgE response against house dust mites (HDM), and this reactivity is directed primarily against the species *Dermatophagoides farinae* (Df). Rates of hypersensitivity to Df reportedly vary between 20 and 100% of atopic dogs.^{1,4}

At the time of writing, 28 Df allergens are included in the official database maintained by the Allergen Nomenclature Subcommittee of the World Health Organization and International Union of Immunological Societies (WHO/IUIS) (www.allergen.org). These are named Der f 1–4, 6–8, 10, 11, 13–18, 20–22 and 24–33. Human patients allergic to *Dermatophagoides pteronyssinus* and *D. farinae* HDM typically do not produce IgE against all of these allergens. Major allergens (i.e. those against which at least 50% of patients allergic to the “parent” allergen source have detectable positive IgE serology or immediate skin test reactivity) are from the group 1 (cysteine protease, 25 kDa), 2 (MD2-like binding protein, 14 kDa) and 23 (peritrophin-like, 8 kDa); these allergens are all present in mite faecal pellets.^{5,6} Interestingly, these

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three major HDM allergens for humans are of low molecular weight (MW). Additional “mid-tier” HDM proteins, against which 20–40% of human mite-allergic patients reportedly are sensitized, are group 4, 5, 7, 13, 15 and 21 *Dermatophagoides* allergens.^{5,6}

The sensitization pattern of atopic dogs to the Df HDM appears quite different from that of allergic humans. In the late 1990s and early 2000s, four publications, two in Europe and two in the USA, reported that dogs hypersensitive to Df HDM recognized predominantly high MW allergens.^{7–11} Further investigations led to the characterization of the high MW Df allergens Der f 15 (98/109 kDa) and 18 (60 kDa); these two allergens are both detectable in the mite digestive tract, but not in their faecal pellets.^{12,13} Importantly, both the Der f 15 chitinase and Der f 18 chitin binding protein were found to be major HDM allergens for dogs, being recognized by IgE from $\leq 100\%$ and 57–80% of dogs hypersensitive to Df, respectively, depending upon the testing method used.^{12,13} In 2007, one of the authors reported, in abstract form, that four Df proteins were major allergens in 144 mite hypersensitive dogs from Japan.¹⁴ Two of these allergens were identified as Der f 2 and 15, whereas another high MW protein (188 kDa; range: 150–250 kDa) was a novel allergen.¹⁴ The latter was provisionally referred to as “Zen-1” and its sequence was deposited in GenBank (<http://www.ncbi.nlm.nih.gov/protein/BAM29295.1>). Further details about this allergen can be found in the US8075898 patent, for example in <https://www.google.com/patents/US8075898>.

Whether or not dogs with AD develop an IgE response to the low MW Df group 1 and 2 allergens remains the subject of controversy. On the one hand, the very low prevalence of IgE reactivity of European and American mite-hypersensitive dogs to the major human mite allergens Der f 1 and 2 was recognized before 2000.^{7–11} On the other, in contrast to these observations, it was reported in 1999 that Japanese atopic dogs had a higher percentage of IgE seropositivity against the low MW group 1 and 2 *Dermatophagoides* allergens than that reported in dogs from the USA and Europe.¹⁵ Indeed, of 16 Japanese atopic dogs with IgE against Df, six (38%) had positive IgE to Der f 1 and seven (44%) binding Der f 2.¹⁵ Other Japanese authors subsequently reported that 44 and 74% of 90 HDM-reactive dogs had IgE antibodies that recognized Der f 1 and Der f 2, respectively.¹⁶ The latter observations suggested that Der f 2 was a major Df allergen for dogs in Japan.

That the *Dermatophagoides* HDM allergome for dogs is very complex was further confirmed by a study testing sera from 20 Portuguese atopic dogs with hypersensitivity to Df and *D. pteronyssinus* (Dp).¹⁷ In that study, which used isoelectric focusing and two-dimensional immunoblotting with Dp (but not Df) proteins, 11 allergens for dogs were identified by the first method and 20 different spots by the second.¹⁷ Interestingly, IgE reactivity to putative group 1 and 2 Dp allergens was found in 16 of 20 (80%) and 10 of 20 dogs (50%), respectively, potentially making these two proteins major allergens for dogs in Portugal.¹⁷

The identification of the Df HDM major allergens for dogs is not only of interest from an evolutionary point of view, but it might also have some significant and clinically

relevant consequences for either standardization of allergen extracts and development of novel allergen-specific immunotherapy protocols. For example, the product Allermune HDM (Nippon Zenyaku Kogyo – Zenoaq; Fukushima, Japan) is an immunotherapy formulation containing high doses of recombinant Der f 2 conjugated with polymers of the maltotriose pullulan. This formulation was found to lead to rapid, robust and persistent improvement in skin lesions and pruritus manifestations in HDM (Der f 2) hypersensitive atopic dogs in Japan.¹⁸

In the present study, we wish to report the IgE reactivity of Df sensitized atopic dogs from the USA and western continental Europe to three purified Df HDM allergens: Der f 1, Der f 2 and Zen-1. We will establish that the novel high MW allergen Zen-1 is a major Df HDM allergen in this species and that it is responsible for a notable percentage of the IgE hypersensitivity to the parent Df HDM. As in most, but not all, previous reports from these countries, Der f 1 and Der f 2 were found to be only minor allergens in dogs hypersensitive to this acarid.

Materials and methods

Blood sample collection and intradermal testing with the purified allergens had been approved beforehand by the Institution's Animal Care and Use Committee.

Canine sera

For this study, we used previously collected sera from dogs with AD diagnosed according to published criteria.¹⁹ In all, there were 33 dogs from North Carolina (USA), 17 dogs from France (Paris or its suburbs) and 12 dogs from Switzerland (Zürich and its surroundings).

We also included sera from 15 laboratory dogs (six beagles, nine Maltese-beagle atopic dogs) sensitized to Df HDM by repeated epicutaneous application of lyophilized ground mites.²⁰ In all of these dogs, hypersensitivity to this allergen had been confirmed beforehand by positive allergen specific intradermal testing (IDT) and/or IgE serology done at regional commercial laboratories. Whether or not these dogs had additional hypersensitivities was not considered relevant to this study.

ELISA

Fluorometric ELISAs for the detection of IgE specific for the crude Df, Der f 1, Der f 2 or Zen-1 were developed specifically for this purpose. Details for the crude Df ELISA can be found in a previous report.²⁰ Although 4.5 μg of Df HDM extract was used to coat the plate for the crude Df ELISA, we used 1 μg of either purified native Der f 1 (Indoor Biotechnologies; Charlottesville, VA, USA), recombinant Der f 2 or native Zen-1 (both from Zenoaq) to coat wells for the three other tests. We had found beforehand that these allergen amounts were optimal for such assay. All sera were initially tested in triplicates at 1:10 and they were diluted further in the case of high reactivity. Successive ELISA steps were identical to those reported earlier.²⁰ For the crude Df serology, the standard curve was created by doubling dilutions of a positive serum control pool (a kind gift from Kenneth Lee, Greer Laboratories; Lenoir, NC, USA). For the standard curves of the other ELISAs, we used sera from dogs experimentally sensitized to Df HDM and for which the previous testing had revealed high IgE reactivity to the individually tested allergens (i.e. Zen-1, Der f 1 or Der f 2); all sera used for standard curves were given a value of 100 units at 1:10 dilution. We expressed results as arbitrary units (AU) and we artificially set the threshold for positivity as the 99% percentile of sera from healthy dogs without skin lesions or pruritus manifestations. These thresholds were 115, 50, 225 and 50 AU for the crude Df, Zen-1, Der f 1 and Der f 2, respectively.

The intra-assay variability of each of the four ELISAs was determined by the testing of five replicates of a low, medium and high reactive serum in the same run. The inter-assay variability was determined by the testing, on six different dates, of the same sera. For both of these, we calculated the coefficient of variability (CV%) as the average of the standard deviation divided by the mean of the testing of the low, medium and highly reactive sera.

Inhibition ELISA

For this test we used sera from 12 dogs with high IgE levels against both the crude Df and Zen-1 and two with IgE recognizing Der f 2. We diluted all sera at 1:400 and incubated them overnight with doubling amounts of Zen-1 (1–16 µg) before testing them by ELISA with plated whole Df, as described above. As a negative inhibitor, sera were incubated with the same amounts of a mix of the Japanese cedar pollen allergens Cry j 1 and Cry j 2 (Zenoag), which are both very rare allergens in the USA. We calculated the ELISA percentage inhibition with 4 µg of the Zen-1 or Cry j 1/2 mix using the following formula: $100 - (\text{OD of serum with inhibitor} / \text{OD of serum without inhibitor}) \times 100$. This amount of inhibitor had been found beforehand to be the lowest allergen concentration to provide maximal inhibition.

Intradermal tests

At a later stage, 25 dogs with AD were tested by IDT at NC State University Veterinary Hospital. In these dogs, which were different from those tested by ELISA, reactivity to Df was tested with intradermal injections of approximately 0.05 mL of 1:1,000 and 1:50,000 dilutions of a commercial crude Df extract (Greer Laboratories). Intradermal tests to Der f 1, Der f 2 and Zen-1 were performed with injections containing 1 µg of the same native purified (Der f 1, Zen-1) or recombinant (Der f 2) allergens that had been used earlier for ELISA. This concentration of allergens had been optimized in prior studies using sensitized dogs. We read urticarial reactions after 20 min; results were considered positive if they were erythematous and of a diameter greater than half that of the positive anti-canine IgE antibody control.²¹

Statistical analyses

For the comparison of proportions of IgE seropositive results to the various allergens or between dog populations, we used the “N-1” chi-square test (https://www.medcalc.org/calc/comparison_of_proportions.php). The correlations between IgE serology AUs for the different allergens were determined by calculation of Spearman’s coefficient (Prism 5.0, Graphpad; La Jolla, CA, USA). For all statistical analyses, the significance threshold was 5% ($P < 0.05$).

Results

ELISA

The ELISAs had an acceptable reproducibility. The intra-assay CV% for the crude Df, Der f 1, Der f 2 and Zen-1 IgE serology were 12.4, 13.2, 10.6 and 10.0%,

respectively. Those for the inter-assay CV% were 10.8, 12.9, 11.0 and 7.5%, respectively. Results for the percentage of IgE seropositivity of canine AD Df reactive sera to the various allergens tested are present in Table 1 and Figure 1. When combining all dogs together, the proportion of IgE reactivity to Zen-1 (86%) was significantly higher than that to the other two allergens Der f 1 (17%) and Der f 2 (19%) (χ^2 test, $P < 0.0001$ for both). There was no significant difference between the prevalence of seropositivity to Der f 1 and that to Der f 2.

As there were no significant differences in the IgE seropositivity to tested allergens between atopic dogs living in France and Switzerland, we analysed these sera collectively in a “European group”. Whereas there was no significant difference in the percentage of IgE seropositivity to Zen-1 between dogs from the three different groups (i.e. American, European and experimentally sensitized), sera from dogs living in the USA were significantly less reactive to Der f 2 than those from the two other groups [χ^2 test, $P = 0.020$ (versus European) and 0.014 (versus sensitized)]. Similarly, American and European dogs were significantly less often seropositive to Der f 1 than those experimentally sensitized to Df (χ^2 test; $P < 0.0001$ and $P = 0.001$, respectively).

Interestingly, the IgE reactivity (AU values) to the crude Df and Zen-1 were highly significantly correlated (Spearman $r = 0.83$, $P < 0.0001$; Figure 2). This observation led us to suspect that a high proportion of the reactivity to the crude Df was due to IgE directed against Zen-1.

Inhibition ELISA

In the 12 sera highly reactive to Zen-1 the percentage of inhibition of Df reactivity by pre-incubation with Zen-1 varied between 22 and 84% (Figure 3; median: 50%). The percentage inhibition with the “negative inhibitor”, a Cry j 1/2 mix, ranged between 0 and 20% (median: 2%; Figure 3). Six of 12 dogs (50%) had Zen-1 inhibitions of Df reactivity superior to 50%. Altogether these results suggested that a substantial portion of the seropositivity to Df was against Zen-1. In the two American dogs with high seropositivity to Der f 2, the percentages of inhibition of crude Der f reactivity by Der f 2 were 32 and 30%, whereas that by Zen-1 was 22% for one of these two sera also having detectable IgE against this allergen.

Table 1. Percentage of IgE serology positivity against *Dermatophagoides farinae* allergens

	Total <i>n</i>	Crude Df (positive threshold: 115 AU)			Der f 1 (positive threshold: 225 AU)			Der f 2 (positive threshold: 50 AU)			Zen-1 (positive threshold: 50 AU)		
		<i>n</i> (%) positive	Median AU	Range AU	% positive	Median AU	Range AU	% positive	Median AU	Range AU	% positive	Median AU	Range AU
Dogs with spontaneous AD													
United States (North Carolina)	33	33 (100%)	524	124–21,862	0 (0%)	0	0–65	2 (6%)	0	0–31,900	29 (88%)	215	0–7,846
Western Continental Europe (France, Switzerland)	29	29 (100%)	4,673	141–32,800	4 (14%)	0	0–9,470	8 (28%)	9	0–7,410	26 (90%)	1,425	0–14,900
Experimentally sensitized dogs (North Carolina)	15	15 (100%)	5,994	227–70,600	9 (60%)	139	0–12,600	5 (33%)	34	0–5,278	11 (73%)	296	0–19,900

DF *Dermatophagoides farinae*, AU arbitrary units, AD atopic dermatitis.

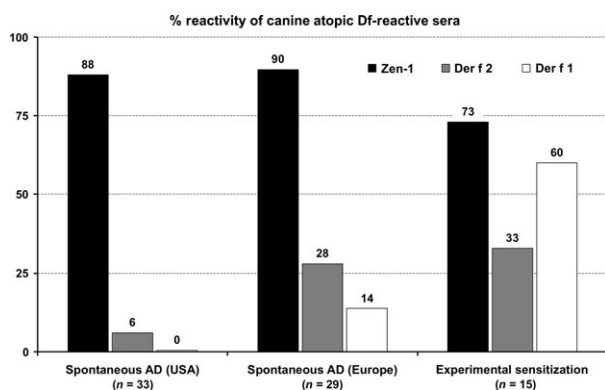


Figure 1. Prevalence of IgE seropositivity to *Dermatophagoides farinae* allergens.

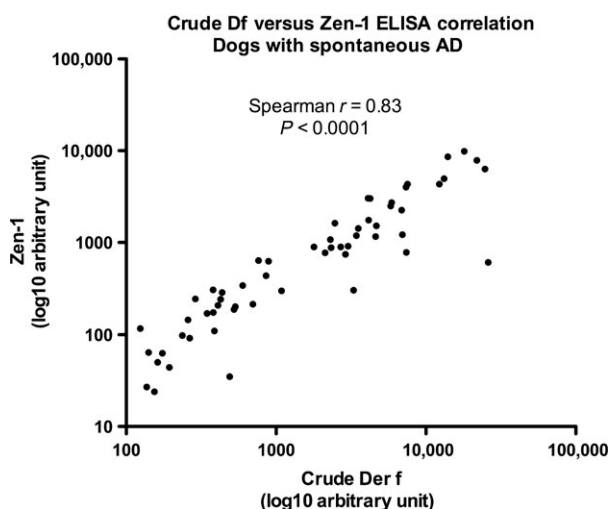


Figure 2. Correlation between IgE seropositivity to the crude *Dermatophagoides farinae* allergen and the novel Zen-1 allergen.

Intradermal testing

Of the 25 atopic dogs tested by IDT, 11 (44%) reacted to both dilutions of the crude Df extract; none responded to the lower dilution (1:1,000) alone. None of the dogs non-reactive to the crude Df extract had any reaction to Der f 1, Der f 2 or Zen-1. Of the 11 dogs reactive to Df, seven (64%), none (0%) and ten (91%) had an immediate positive reactions to Der f 1, Der f 2 and Zen-1, respectively.

Discussion

Our results establish that most dogs with positive IgE serology or IDT to Df have detectable IgE directed against the novel high MW 150–250 kDa allergen provisionally named Zen-1. The sole reactivity to this allergen appears to represent, in most dogs tested, a majority of the seropositivity to the parent allergen. In contrast to this observation, the reactivity to the low MW allergens Der f 1 and 2 was less common in the dogs tested herein; it was variable between dog groups, being highest in dogs experimentally sensitized to Df.

A downside of our study was the lack of IgE serology performed with the two previously high MW allergens Der f 15 and 18,^{12,13} but these allergens were not available, in either purified or recombinant form, at the time our testing was completed. Another limitation is that IgE serology

tests and IDT were not done on the same mite-reactive atopic dogs, because the serum testing used archived samples and we performed the IDT at a later date on client owned pets. Nevertheless, in spite of this difference in tested dogs, the prevalence of positive IgE serology and IDT [both done with similar amounts (i.e. 1 µg) of the Zen-1 protein] were nearly identical and high. These results provide evidence that Zen-1 is, indeed, a major allergen in European and American atopic dogs reactive to Df.

At the time of writing little is known about the Zen-1 protein and its function; its sequence (<http://www.ncbi.nlm.nih.gov/protein/BAM29295.1>) is very different from that of other known Df or Dp allergens. In spite of this difference, there are several pentameric proline- and threonine-rich repeats (PTTPT) in the middle of the Zen-1 sequence that align with similar repetitive ones in the carboxy terminal third of Der f 15. Although the 3D folding of Zen-1 is not known, it is conceivable, at least theoretically, that it might share surface conformational epitopes with Der f 15 in the areas of these pentameric amino acid repeats; such conformation would make these two allergens cross-reactive on peptide epitopes.

Interestingly, a further analysis of the sequence of Zen-1 suggests that this protein is highly O-glycosylated on the numerous proline and threonine residues repeats in its middle (amino acids 116–288) and carboxy terminus (amino acids 379–473) (NetOGlyc 4.0; <http://www.cbs.dtu.dk/services/NetOGlyc/>). This observation is similar to the O-glycosylation expected in the carboxy terminal region (amino-acids 413–498) of Der f 15.^{1,12} Importantly, IgE epitopes for Der f 15 seem located on these O-glycans, as the enzymatic and chemical removals of its amino acids do not reduce much canine IgE binding. In contrast N-glycans appear absent from this allergen (Patent US7128921; <http://www.google.com/patents/US7128921>). As a result of these glycosylation predictions, it is conceivable that Zen-1 and Der f 15 could be hypothetically cross-reactive via O-linked carbohydrate determinants. These cross-reactive O-glycans would likely be pathogenic because of the high prevalence of positive immediate IDT reactions existing with these two allergens in dogs. The theoretical possibility of Zen-1 cross-reactivity to Der f 15, because of similarities in either peptide and/or O-glycan IgE epitopes, must be verified by inhibition ELISA with intact and deglycosylated allergens. Indeed such cross-reactivity could have important and clinically relevant implications for the standardization of allergen extracts, as well as for immunotherapy purposes.

In our study with sera of Df-reactive atopic dogs from Europe and the USA, the prevalence of positive IgE serology to Der f 1 and Der f 2 was either very low (Der f 1), or low and variable (Der f 2). These results mirror those of previous testing in European and American dogs, studies that used either purified group 1 and 2 allergens,^{7,9} or whole mite extracts.^{7,9,10} In contrast to these early observations, there are higher Der f 1 and Der f 2 IgE seropositive reactions in mite allergic dogs in Japan.^{15,16} That Der f 2 is an important and relevant mite allergen in Japanese atopic dogs is also supported by the rapid and robust response of Df/Der f 2-reactive dogs to immunotherapy performed with high doses of the single and pullulan-conjugated allergen Der f 2.¹⁸ An unexplained discrepancy in

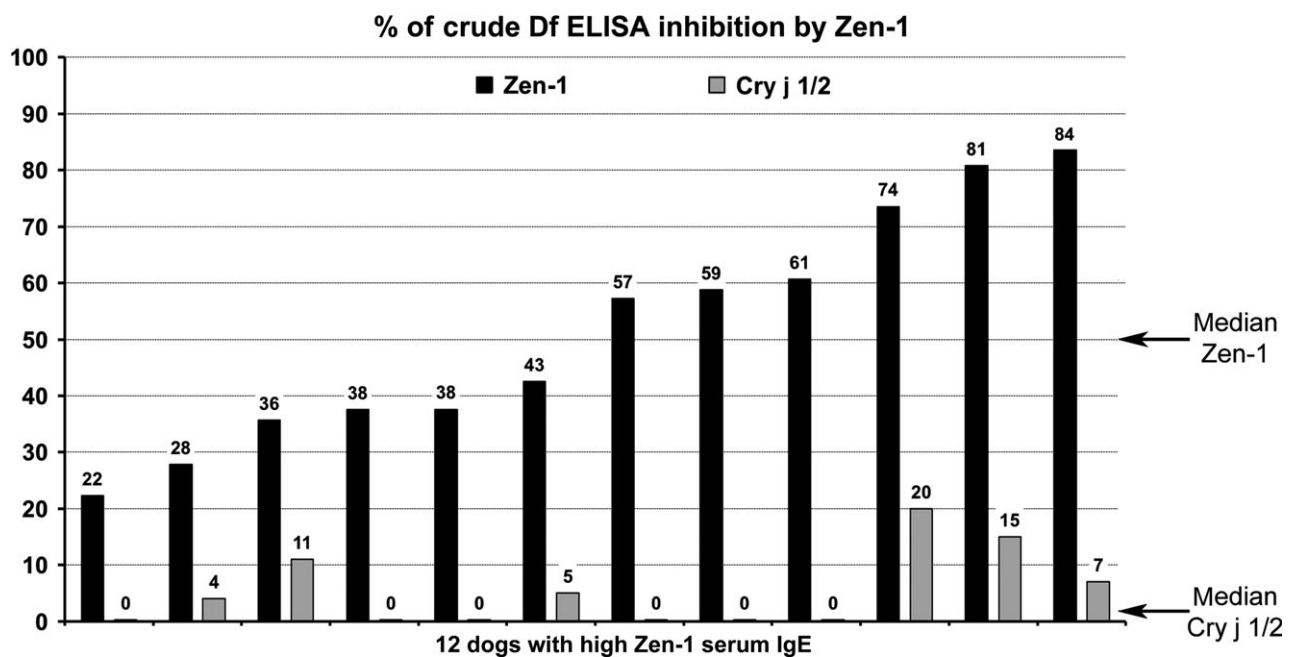


Figure 3. Inhibition of crude *Dermatophagoides farinae* ELISA after pre-incubation of sera with Zen-1 and Cry j 1/2.

our study was the lack of IgE seropositivity of American dogs to Der f 1, whereas two thirds of the Df-sensitized dogs from the same geographical area had an IDT response to the same amount (1 µg) of this protein. This discrepancy was present even though we used the same manufacturing source for allergens for both ELISA and IDT. A nonspecific (i.e. "irritant") reaction seemed unlikely, as none of the 14 dogs that did not have an immediate IDT response to crude Df, also reacted to this allergen.

Why there is such a discrepancy in the IgE reactivity to the low MW group 1 and 2 Df allergens between western and Japanese dogs could involve a combination of differences in both genetic, geographical and environmental factors. For example, it is theoretically possible that a difference in class II major histocompatibility complex alleles between western and Asian dogs could result in variable allergenic epitope presentation by antigen presenting cells to T cells. This theory is, however, hard to conceive because this IgE recognition of Der f 2 in Japan is present in dogs from several different breeds, and the variability in MHC II alleles would have to affect most, if not all, of these breeds that have a different ancestry.

Geographical differences in both mite prevalence as well as in *Dermatophagoides* allergenic sequences have been shown.^{5,22} Although Der f 1 is not very polymorphic in its sequence,^{5,23} amino acid substitutions in Der f 2 seem to reflect genetic evolution. Indeed, the sequences of Der f 2 from Asia and Europe appear to belong to different groups. For example, although two thirds of German Df have the Der f 2.0107 variant and one third has Der f 2.0101, Japanese Df has mostly Der f 2.0101.^{22,23} Similarly, Der f 2.0102 and Der f 2.0116 represent 41% and 6%, respectively, of Der f 2 sequences in Korea,²⁴ whereas the latter is mentioned to be more prevalent in Europe.⁵ It is plausible, although unproven, that sequence variations in Der f 2 could result in dogs becoming variably sensitized to this allergen if they lived in markedly different geographical locations. This hypothesis would have to

be reconciled with the polyclonal IgE response that is typically mounted against an allergen, thereby making single polymorphisms in allergen sequence less susceptible to influence the overall reactivity to the whole allergen, unless these polymorphisms markedly altered the structure of allergenic epitopes.

In support for this last hypothesis, genetic polymorphisms of *Dermatophagoides* group 2 allergens (for example at amino acid 114 in Der p 2) appear to influence their immunogenicity,^{25,26} as sequence variants cluster in a region accessible to antibody binding.^{25,27} Furthermore, human serum IgE binds to Der p 2.0104 at higher titres than to Der p 2.0101.²⁷ In spite of these observed geographical variations in the sequence of Der f 2 isoallergens, a seroepidemiological survey of over 1,300 *Dermatophagoides*-sensitized human patients confirmed a similar rate of IgE seropositivity to Der f 2 in Europe, Canada, the USA and Japan.⁶ As a result, differences in the worldwide prevalence in the various Der f 2 isoallergens might have little influence on the overall IgE reactivity to this allergen in human mite-sensitized patients. Whether or not they could have in dogs is unknown, but it does not appear very likely.

Alternatively and finally, differences in sensitivities between testing methods (immunoblotting versus ELISA) or between ELISAs using differing methodologies could affect the rate of detection of Der f 2 IgE seropositivity. Consequently, global serological surveys using the same assay are warranted to confirm if differences in the Der f 2 IgE reactivity do exist in dogs living in different countries, continents or climates.

In contrast to the situation seen in American dogs with spontaneous AD, dogs experimentally previously sensitized to Df mounted IgE responses against this mite's group 1 and 2 allergens. It is possible that our "un-natural" sensitization method (i.e. repeated epicutaneous applications of a high amount of a lyophilized Df HDM suspension in mineral oil)²⁰ might trigger an excessive

exposure to the group 1 and 2 Df allergens that does not occur in a natural environment. However, that such Der f 1- and 2-specific IgE could occur after sensitization with mites cultured in the USA does somewhat invalidate the hypothesis of differences in sensitization occurring due to geographical variations in allergen sequence polymorphisms. At this time, the particular reason(s) for this difference in the sensitization prevalence to Der f 1 and 2 between atopic dogs from Japan and Western countries is unknown, but this topic is clearly deserving of further studies because of the possible implications for allergen testing and allergen specific immunotherapy.

In conclusion, our results suggest that, in both mite-reactive atopic dogs from Europe and the USA, the novel high MW allergen Zen-1 is a major and important Df allergen. In contrast, Der f 1 and Der f 2 appear to be minor mite allergens in dogs from these locations. Further studies, with a larger number of canine sera from these and additional geographical areas, should be performed with the same serological assay to verify the validity of these findings.

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Note

After acceptance of this paper, an article²⁸ reported ELISA and immunoblotting evidence that both Der f 1, Der f 2, Der f 15 and Der f 18 were all major allergens in atopic dogs sensitized to the *Dermatophagoides farinae* house dust mites.

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Résumé

Contexte – Les chiens atopiques hypersensibles à Df (*Dermatophagoides farinae*) possèdent des IgE reconnaissant des allergènes de haut poids moléculaire (MW) plus souvent que Derf 1 et 2 de faible MW. Un nouvel allergène de haut MW, Zen-1, a récemment été identifié.

Objectifs – Déterminer la réactivité des IgE des chiens hypersensibles américains et européens à Zen-1, Derf 1 et Derf 2.

Méthodes – Nous avons testé les séras de 33 chiens réactifs à Df venus des USA, 29 venant d'Europe et 15 expérimentalement sensibilisés à Df, par ELISA contre Df brut, Derf 1, Derf 2 et Zen-1. L'inhibition d'ELISA a été réalisée par des séras réactifs à Zen-1. Les tests intradermiques (IDT) ont également été réalisés avec les mêmes allergènes chez 25 autres chiens atopiques américains.

Résultats – Au total, la séropositivité des IgE à Zen-1 était plus prévalente (86%) qu'à Derf1 (17%) ou Derf 2 (19%). La réactivité des IgE à Zen-1 était corrélée à celle de Df brut; cet allergène seul inhibe un pourcentage élevé (médiane : 50%; écart : 22–84%) du portage à l'extrait brut d'acararien. La séropositivité aux allergènes de faible MW était plus élevée chez les chiens sensibilisés expérimentalement. La connaissance d'IgE sériques de Derf 1 était faible chez les chiens atopiques; celle à Derf 2 était significativement plus faible chez les chiens américains (6%) qu'européens (28%). Une haute prévalence de réactions positives immédiates à Zen-1 a confirmé la probable prévalence des résultats sérologiques.

Conclusions et importance clinique – Cette étude met en évidence que Zen-1 est un allergène majeur chez les chiens atopiques sensibilisés à Df.

Resumen

Introducción – perros atópicos con hipersensibilidad a *Dermatophagoides farinae* (Df) tienen IgE frente a alérgenos de alto peso molecular (MW) con más frecuencia que frente a los de bajo peso molecular Der f 1 y 2. Un nuevo alérgeno Df de alto PM Df, Zen-1, ha sido identificado recientemente.

Objetivos – Determinar la reactividad de IgE de perros americanos y europeos con hipersensibilidad a Df frente a Zen-1, Der f 1 y Der f 2.

Métodos – Se probaron los sueros de 33 perros reactivos a Df procedentes de los EE.UU., 29 de Europa y 15 sensibilizados de forma experimental frente a Df, por técnica de ELISA con extracto crudo de Df, Der f 1, Der f 2 y Zen-1. Un ELISA de inhibición se realizó con sueros reactivos a Zen-1. Las pruebas intradérmicas (IDT) se hicieron también con los mismos alérgenos en otros 25 perros atópicos estadounidenses.

Resultados – En total, la seropositividad de IgE frente a Zen-1 fue más frecuente (86%) que la encontrada frente a Der f 1 (17%) o Der f 2 (19%). La reactividad de IgE a Zen-1 se correlacionó con la hallada frente a extracto Df crudo; este alérgeno por sí sólo inhibió un alto porcentaje (mediana: 50%; intervalo: 22 a 84%) de la unión al extracto de ácaro crudo. La seropositividad a los alérgenos de bajo PM fue mayor en los perros experimentalmente sensibilizados. El reconocimiento de IgE sérica frente a Der f 1 fue baja en los perros con AD; la presencia de IgE frente a Der f 2 fue significativamente menor en los perros americanos (6%) que en el resto de Europa (28%). Una alta prevalencia de las reacciones inmediatas IDT positivas a Zen-1 confirmó la relevancia de los resultados serológicos.

Conclusiones e importancia clínica – Este estudio establece Zen-1 como un alérgeno importante en los perros atópicos sensibilizados a Df.

Zusammenfassung

Hintergrund – Atopische Hunde mit einer Hypersensibilität auf *Dermatophagoides farinae* (Df) haben häufiger durch IgE erkannte hochmolekular gewichtige (MW) Allergene als niedrig molekulare Der f1 und 2. Ein neues hochmolekulares MW Df Allergen Zen-1, wurde unlängst identifiziert.

Ziele – Die Bestimmung der IgE Reaktivität von amerikanischen und europäischen Df-hypersensiblen Hunden auf Zen-1, Der f 1 und Der f 2.

Methoden – Wir überprüften Sera von 33 Df-reaktiven Hunden aus den USA, 29 aus Europa und 15 experimentell zu Df sensibilisierten Hunden mittels ELISA gegen grobes Df, Der f 1, Der f 2 und Zen-1. ELISA Inhibition wurde mit Sera, die auf Zen-1 reagierten durchgeführt. Intradermaltests (IDT) wurden ebenfalls mit denselben Allergenen bei 25 anderen amerikanischen atopischen Hunden durchgeführt.

Ergebnisse – Insgesamt war eine Seropositivität von IgE auf Zen-1 häufiger (86%) als jene auf Der f 1 (17%) oder Der f 2 (19%). Die IgE Reaktivität auf Zen-1 wurde mit jener auf das grobe Df korreliert; dieses Allergen inhibierte alleine einen großen Prozentsatz (Median: 50%; Breite: 22–84%) der Bindung durch den groben Milbenextrakt. Die Seropositivität zu niedrig molekularen MW Allergenen war bei den experimentell sensibilisierten Hunden am niedrigsten. Die IgE Erkennung von Der f 1 im Serum war niedrig bei Hunden mit AD; jene zu Der F 2 war signifikant niedriger bei den amerikanischen Hunden (6%) als bei den europäischen (28%). Eine hohe Prävalenz von positiven IDT Sofortreaktionen zu Zen-1 bestätigte die wahrscheinliche Relevanz der serologischen Ergebnisse.

Schlussfolgerungen und klinische Bedeutung – Diese Studie etabliert Zen-1 als Hauptallergen bei atopischen Hunden, die auf Df sensibilisiert sind.

要約

背景 — *Dermatophagoides farinae*(Df)に過敏症を示すアトピーの犬は、低分子(MW)アレルゲンであるDer f 1や2よりも、高MWアレルゲンを認識するIgEをより高頻度に保有する。近年、新規の高MW DfアレルゲンであるZen-1が同定された。

目的 — アメリカおよびヨーロッパのDf過敏症の犬における、Zen-1、Der f 1およびDer f 2に対するIgEの反応性を調べること。

方法 — USAからの33検体、ヨーロッパからの29検体、および、実験的にDfに感作させた15検体のDf反応性の犬の血清を用いて、未加工のDf、Der f 1、Der f 2およびZen-1についてELISAにて検査した。Zen-1に対する血清の反応性についてのELISA阻害を実施した。別のアメリカのアトピー犬25頭を用いて、同じアレルゲンに対する皮内試験(IDT)も実施した。

結果 — 全体的に、Zen-1に対するIgE血清陽性は(86%)、Der f 1(17%)あるいはDer f 2(19%)よりも高頻度に認められた。Zen-1に対するIgE反応性は未加工のDfに対するものと相関していた。このアレルゲンは、未加工のダニ抽出液に対する結合を、高い確率で阻害した(中央値: 50%; 範囲: 22–84%)。低MWアレルゲンに対する血清陽性は、実験感作犬が最も高かった。血清IgEのDer f 1認識は、ADの犬で低く、Der f 2のそれは、アメリカの犬(6%)でヨーロッパの犬(28%)よりも有意に低かった。IDTでのZen-1に対する高い即時型反応陽性率は、血清試験の結果の妥当性を裏付けた。

結論および臨床的重要性 — 本研究において、Zen-1がDfに感作されたアトピー犬の主要アレルゲンであることを証明した。

摘要

背景 — 粉尘螨(Df)過敏の异位性犬、对高分子量的過敏原(MW)比低分子量的 Der f1和f2更易产生相应的IgE识别抗体。而最近发现了一种新型的高MW Df過敏原,Zen-1。

目的 — 确定美国和欧洲地区Df過敏犬对Zen-1,Der f1和Der f 2的IgE反应特性。

方法 — 我们对来自美国的33个,来自欧洲的29个Df-反应性犬,和15个实验性致敏Df的犬血清,用ELISA的方法,对天然Df,Der f 1,Der f 2和Zen-1进行测试。ELISA 抑制试验检测Zen-1的血清学反应。对25只来自美国的异位性犬,使用相同的過敏原进行皮内试验(IDT)。

结果 — 总体上,Zen-1的IgE血清(86%)比Der f 1(17%)或Der f 2(19%)呈现更高比例的阳性结果。IgE对Zen-1的反应性,与其对天然Df的反应相关;与天然螨提取物结合状态下,这种過敏原会很高比例地(中值:50%;范围:22–84%)被单独抑制。实验致敏犬对低分子量MW過敏原产生的血清阳性比例最高。在AD患犬中,识别Der f 1的血清IgE水平低;而对于Der f 2来说,美国犬(6%)显著低于欧洲犬(28%)。对Zen-1的阳性即时IDT反应的高发生率,确认了可能与血清学结果相关。

结论和临床重要性 — 这项研究确认,异位性犬对粉尘螨的主要過敏原是Zen-1。

Resumo

Contexto – Cães atópicos com hipersensibilidade a *Dermatophagoides farinae* (Df) possuem mais frequentemente IgE que reconhecem alérgenos de alto peso molecular que os específicos para Der f 1 e f 2, que são de baixo peso molecular. Um novo tipo de alérgeno de Df de alto peso molecular, Zen-1, foi identificado recentemente.

Objetivos – Determinar a reatividade de anticorpos IgE de cães americanos e europeus Df-hipersensíveis aos antígenos Zen-1, Der f 1 e Der f 2.

Métodos – Foram testadas amostras de soro de 33 cães Df-hipersensíveis oriundos dos EUA, 29 da Europa e 15 Df-hipersensíveis sensibilizados experimentalmente. Os antígenos utilizados para ELISA foram Df cru, Der f 1, Der f 2 e Zen-1. Inibição de ELISA foi realizada com soro reativo a Zen-1. Teste intradérmico (IDT) também foi realizado com os mesmos alérgenos em outros 25 cães atópicos americanos.

Resultados – Soropositividade a Zen-1 foi mais prevalente (86%) que a Der f1 (17%) e Der f2 (19%). A reatividade de IgE a Zen-1 correlacionou-se com àquela contra o Df cru. Zen-1 isoladamente inibiu uma alta porcentagem (mediana: 50%; intervalo: 22–84%) da ligação ao extrato cru de ácaro. A soropositividade a antígenos de baixo peso molecular foi maior em cães sensibilizados experimentalmente. O reconhecimento de IgE sérica a Der f 1 foi baixo em cães com dermatite atópica; a soropositividade a Der f 2 foi significativamente menor em cães americanos (6%) que nos europeus (28%). Alta prevalência de reações positivas imediatas a Zen-1 em IDT confirmou a relevância provável dos resultados sorológicos.

Conclusões – Este estudo demonstra que Zen-1 é o principal alérgeno em cães atópicos sensibilizados contra Df.