Veterinary Dermatology

*Vet Dermatol* 2016 DOI: 10.1111/vde.12393

# 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

Accepted 23 September 2016
**Sources of Funding:** This study was funded by a grant of the Zenoaq Corporation to NC State University, this included contributions to consumables and provision of allergens and technical support.
**Conflict of Interest:** Toshirh Tsukui is an employee of the company sponsoring this project.
**Previous Presentation:** This study was published, in part, as an abstract of the North American Veterinary Dermatology Forum, 2009, Savannah, GA. *Vet Dermatol* 2009; 20: 215 (abstract).

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 ≤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 Allermmune 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.