

Allergen components of *Dermatophagoides farinae* recognised by serum immunoglobulin (Ig)E in Thai dogs with atopic dermatitis

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Background – *Dermatophagoides farinae* (Der f) is a common allergen in dogs with atopic dermatitis (AD). However, the relevant components of Der f require further investigation.

Objectives – We aimed to provide data on the immunoglobulin (Ig)E-binding specific components of Der f for further diagnostic and therapeutic applications.

Animals – Serum samples were collected from five healthy, nine Der f-allergic atopic and seven non-Der f-allergic atopic dogs identified based on an intradermal skin test.

Methods and materials – We explored the component profiles of Der f extracts through 2D gel electrophoresis and IgE immunoblotting. The IgE-binding components in both groups of atopic dogs were analysed by mass spectrometry.

Results – The majority of Der f-allergic atopic dogs recognised Der f *Alternaria alternata* allergen 10 (Der f Alt a 10), elongation factor 1- α (EF1- α), gelsolin-like allergen Der f 16, Der f 28 and Der f 2. Der f 3, Der f 10, Der f 20 and Der f 32 were recognised as minor allergens. Alpha-enolase, serine protease, arginine kinase and a few hypothetical proteins were recognised components in both groups of atopic dogs. Unexpectedly, Der f 15 (chitinase) was found to be a minor component.

Conclusions and clinical importance – Multiple IgE-binding allergens of Der f were identified in Thai atopic dogs. We propose that the specific antigen set that is bound by IgE, comprising Der f Alt a 10, EF1- α , gelsolin-like Der f 16, Der f 28 and Der f 2, could be used for future diagnostics and immunotherapy platforms.

Introduction

Atopic dermatitis (AD) in dogs is a genetically predisposed inflammatory and pruritic skin disease which is associated with multiple factors.¹ From the prevalence revealed from allergen testing in different regions, house dust mites (HDM) act as a dominant allergen, especially the HDM *Dermatophagoides farinae* (Der f).^{2–4} Allergen testing or allergen-specific immunotherapy (ASIT) in dogs

still uses crude HDM extracts, which contain varied concentrations of allergenic and nonallergenic components.⁵ In atopic human patients, *Dermatophagoides pteronyssinus* allergen 1 (Der p 1), Der f 1, Der p 2, *Felis domesticus* allergen 1 (Fel d 1), *Canis familiaris* allergen 1 (Can f 1), *Rattus norvegicus* allergen 1 (Rat n 1), *Mus musculus* allergen 1 (Mus m 1) and *Blattella germanica* allergen 2 (Bla g 2) have been combined as an “universal” allergen standard (UAS), and used in a fluorescent multiplex array to detect serum reactivity to indoor allergens.⁶ For atopic human patients who are sensitive to Der f, a purified Der f 1 allergen has been included as a standardised allergen in commercial extracts for decades. The type and concentration of the reference standard used for immunotherapy is based on guidelines for exposure levels that result in allergic sensitisation or symptom exacerbations. By contrast, for dogs there is no consensus on the set of commercial Der f allergens to be used, owing to an

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incomplete definition of the major allergen component of Der f in atopic animals.^{7,8}

In clinical practice, allergen-specific immunoglobulin (Ig)E levels are used to support the diagnosis of AD in allergic humans and dogs.^{1,9} Furthermore, although the symptoms of allergy may be mitigated by pharmacological treatment, only ASIT can prevent disease progression, with sustainable effects.¹⁰ Identification of mite allergen reactions in atopic dogs is essential for improved diagnosis and treatment. Component-resolved diagnosis (CRD) has been developed to designate appropriate diagnostic platforms based on pure allergen molecules which are produced either by recombinant expression of allergen-encoding cDNAs, or by purification from natural allergen sources.¹¹ This approach also is beneficial in preparing immunotherapy formations, in a technique called component-resolved immunotherapy (CRIT).¹¹ Previously, a single recombinant Der f 2 allergen conjugated with pullulan has been used in an immunotherapy formulation for atopic dogs, where it relieved clinical signs and reduced pruritus levels within two months of treatment.¹² Currently it is important to understand reactivity to specific local mite-allergens in atopic dogs in our area.

From the Database on Allergen Nomenclature, 35 components involved in Der f-related allergic disease in humans have been defined. Of these, Der f 1, Der f 2, Der f 10, Der f 11, Der f 14, Der f 15, Der f 16, Der f 18, Der f 25, Der f 29 and Der f 30 have been reported as the major allergens in allergic human patients. Knowledge of the types and prevalence of allergens can enhance understanding of the pathogenesis as it relates to the biological activity of the allergen. The prevalence of IgE binding to Der f components is still poorly defined and classified in atopic dogs. Previous studies found that Der f 2, Der f 15 and Der f 18 were significantly recognised by most atopic dogs, while Der f 1 was less commonly recognised. Importantly, the prevalence of IgE-binding to each Der f component varied depending on the geographical region where the study was conducted.^{13–17} Recently, a novel allergen called “Zen-1” was established as a major allergen in North American and European atopic dogs sensitised to Der f.¹⁸ However, other components of Der f have not been explored and identified in dogs, especially atopic dogs in Thailand.

Thus, the aim of this study was to explore and identify components of Der f by using 2D electrophoresis (2DE) and undertaking proteomic analysis of the IgE-recognised components of Der f in Thai atopic dogs.

Methods and materials

Serum samples

This study protocol was approved by the Institution Animal Care and Use Committee of Chulalongkorn University Animal Care and Use Protocol; No. 1731046. Serum samples were collected from five healthy dogs and 16 dogs with AD from the Veterinary Teaching Hospital of Kasetsart University and Small Animal Teaching Hospital of Chulalongkorn University. All healthy dogs were enrolled with the inclusion criteria determined by a normal physical examination, to include not suffering from any diseases, and having no previous history or current clinical signs of skin problems. The atopic dogs were recruited following the guideline for canine AD diagnosis according to the International Committee for Allergic Diseases in Animals

(ICADA).¹ Before study enrolment, putative atopic dogs must have been subjected to empiric therapy against ectoparasitic infestation for at least three months. Skin scrapings, hair pluckings and ear swabs were examined to rule out ectoparasitic infestation. Combined with a prior history of ineffective antimicrobial treatment for their condition, and cytological samples not showing significant findings, both pyoderma and *Malassezia* dermatitis were ruled out. Subsequently, a strict elimination diet was fed to the dogs for at least eight weeks to eliminate cutaneous adverse food reactions as a cause of dermatitis. Where clinical signs remained after the diet trials, dogs were diagnosed as having AD. Intradermal testing (IDT) of 47 selected local aero-allergens was performed on the dogs, as reported previously.² All anti-inflammatory and antipruritic drugs were withdrawn at least two weeks before testing. Locally sourced Der f extracts and Der p extracts were obtained from The Siriraj House Dust Mite Center for Services and Research, and were used at the dilution recommended by the manufacturer.^{2,19} Skin reactions were evaluated 15 min after injection. To be considered positive, the diameter and redness of the wheal at the injection site had to be greater than or equal to the mean of the diameter and redness of the wheal at sites injected with histamine and buffered saline. All serum samples were collected and stored at –20°C until used.

Preparation of Der f extracts

Two grams of purified Der f with >99% purity from Siriraj House Dust Mite Center for Services and Research was washed three times and resuspended in 8 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4). The suspension was homogenised in an Omni Sonic Ruptor 4000 (Omni International; Kennesaw, GA, USA) at 35% amplitude with 0.5 cycles (15 min, on ice) and the supernatant was collected after centrifugation at 12,000g, at 4°C, for 5 min (Sorvall Legend X1R, Thermo Fisher Scientific; Waltham, MA, USA). The protein concentration in the supernatant was measured using the Protein Assay Kit II (Bio-Rad; Hercules, CA, USA) and was kept in aliquots at –20°C until used.

Antigen analysis by 2DE and mass spectrometry

The Der f extract was cleaned to eliminate nonprotein contaminants using a 2D-Clean up kit (GE Healthcare; Tyrone, PA, USA), and the protein concentration was determined using a 2D-Quant kit (GE Healthcare). One hundred microgrammes of Der f extract were added to the DeStreak Rehydration Solution containing 0.5% (w/v) IPG buffer pH 3–11 nonlinear (GE Healthcare) and transferred to a strip holder. An IPG strip (Immobiline DryStrip pH 3–10 NL, 7 cm, GE Healthcare) was placed into the strip holder, and then covered with rehydration solution cover fluid at 25°C overnight before running the 2DE. The running protocol for the first dimensional isoelectric focusing electrophoresis was an initial 0.2 kV/h for 30 min, a gradient step at 0.3 kV/h for 30 min, 4.5 kV/h for 90 min and a holding step for 30 min. The focused IPG strip was transferred to reduction buffer [50 mM Tris-HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) bromophenol blue and 1% (w/v) dithiothreitol (DTT)] at 25°C for 15 min and then placed into alkylation buffer [50 mM Tris-HCl pH 8.8, 6 M urea, 30% (v/v) glycerol, 2% (w/v) SDS, 0.002% (w/v) bromophenol blue, and 2.5% (w/v) iodoacetamide] for 15 min. The second dimension was performed by 12% (w/v) polyacrylamide gel electrophoresis and the resolved protein spots were revealed by silver staining.

IgE immunoblotting

The 2DE separated Der f extract was blotted onto a nitrocellulose membrane (0.45 µm pore, Thermo Fisher Scientific) and the protein spots were stained by Ponceau S staining. The membrane was washed with PBST washing buffer [PBS containing 0.05% (v/v) tween 20] and then soaked in blocking buffer [5% (w/v) skimmed milk in PBST] for 1 h. After washing, the membranes were incubated with individual serum samples from atopic dogs (diluted 1:5 in blocking buffer) at 4°C overnight. In the case of healthy dogs, five sera samples were pooled before use. The membrane was incubated for 2 h with diluted 1:2,000 mAb mouse anti-dog IgE (clone E6-2A1,

AbD serotec (Bio-Rad); Oxford, UK) in blocking buffer. The membrane was washed and incubated with goat anti-mouse IgG conjugated to horseradish peroxidase (HRP) diluted 1:4,000 in blocking buffer (Southern Biotech; Birmingham, AL, USA) for 1 h. After the final wash, the membrane was reacted with SuperSignal West Pico (Thermo Fisher Scientific) and visualised using the gel doc system (G:BOX Chemi, Syngene; Cambridge, UK).

Protein identification by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS).

For spot selection, the common spots were culled out and the unique spots, which were visualised among the membranes from atopic dogs, were selected for identification using LC-MS/MS. The silver-stained protein spots, corresponding to those spots visualised on the membranes, were excised from the gel and destained by incubating for 30 min in 30 mM potassium ferricyanide [$K_3Fe(CN)_6$; Merck; Kenilworth, NJ, USA] and 100 mM sodium thiosulfate (Merck) solution. For digestion, gel pieces were reduced with 4 mM dithiothreitol (DTT) at 60°C for 15 min and were alkylated in 250 mM iodoacetamide at room temperature for 30 min. The gel pieces were dehydrated with 100% (v/v) acetonitrile (ACN) (Thermo Fisher Scientific) and digested by trypsin (T6567, Sigma-Aldrich; Dorset, UK) overnight at 37°C. The peptides were extracted by adding ACN and incubating for 20 min. The samples were stored at –20°C until analysis. All protein samples were analysed using an Ultimate 3000 Nano-LC system (Thermo Fisher Scientific) coupled with a microTOF-Q II (Bruker Daltonics; Bremen, Germany), both controlled by HyStar v3.2 (Bruker; Bremen, Germany).²⁰ For selection, MS and MS/MS spectra covered mass ranges of m/z 400–2,000 and m/z 50–1,500, respectively. For identification, the raw data format files were processed and converted to mascot generic files using COMPASS DATAANALYSIS software v3.4 (Bruker) and screened for matches against the *Dermatophagoides* database and insect database using MASCOT DAEMON software (Matrix Science; Boston, MA, USA). Only proteins identified with the highest scores were reported.

Results

Characteristics of the study samples

Regarding the dog's history and IDT results, the 16 atopic animals were classified into nine with a positive IDT to

Der f (AD + IDT to Der f) and seven with a negative IDT to Der f (AD – IDT to Der f). Breed, sex and age varied in each group of dogs, as shown in Table 1.

2DE pattern of the Der f extracts and Der f-specific IgE spots in dogs

After silver staining, the representative 2DE pattern is shown in Figure 1. Only 43 spots of Der f extract were recognised by IgE. In each group and in individual dogs, the IgE-binding spots differed in their number and pattern of recognition (Figure 2). However, nine IgE-binding spots were found to be common in all groups, and these were >30 kDa. A total of 34 specific IgE-binding spots from both groups of atopic dogs were selected for the further protein identification using LC-MS/MS analysis. After identification, 14 IgE-binding components were found in both the AD + IDT to Der f and AD – IDT to Der f groups, as shown in Figure 3. With respect to the unique IgE-binding components in each group of atopic dogs, 10 IgE-binding components were found only in the AD + IDT to Der f group, while one IgE-binding component was found in the AD – IDT to Der f group.

Regarding the LC-MS/MS data, the type of allergen components/proteins, and frequency and percentage of recognition are shown in Table 2, while the accession number of the sequence and biological function of each respective protein spot is summarised in Table 3. At least half of the serum samples from atopic dogs in each group bound to alpha-enolase, serine protease, arginine kinase and to two different types of hypothetical proteins (spots 6 and 22). In the AD + IDT to Der f group, Der f *Alternaria alternata* allergen 10 (Der f Alt 10), elongation factor 1- α (EF1- α), gelsolin-like allergen Der f 16, Der f 28 and Der f 2 (spot 34), were the dominant components only for this group. By contrast, other components, such as Der f 2 (spot 1), Der f 3, Der f 10, Der f 20, Der f 32, three hypothetical proteins (spots 11, 21 and 24), and an unnamed protein product (spot 28), were minor IgE-binding

Table 1. Characteristics of serum samples from dogs¹⁶

Subject	Age (years)	Sex	Breed	Clinical diagnosis	IDT to Der f	Positive IDT to other allergens
H1	2	F	Rottweiler	Non-AD	N/A	N/A
H2	4	F	Siberian husky	Non-AD	N/A	N/A
H3	5	M	Beagle	Non-AD	N/A	N/A
H4	3	F	Rottweiler	Non-AD	N/A	N/A
H5	3	F	Rottweiler	Non-AD	N/A	N/A
AD1	13	M	Poodle	AD	+	–
AD2	11	F	Cross-breed	AD	+	<i>Dermatophagoides pteronyssinus</i>
AD3	1	M	West Highland white terrier	AD	+	<i>D. pteronyssinus</i>
AD4	1	F	French bulldog	AD	+	<i>D. pteronyssinus</i>
AD5	15	M	Shih tzu	AD	+	<i>D. pteronyssinus</i>
AD6	4	M	Beagle	AD	+	<i>Periplaneta americana</i>
AD7	3	M	Beagle	AD	+	<i>D. pteronyssinus</i>
AD8	3	F	Beagle	AD	+	<i>D. pteronyssinus</i>
AD9	8	F	Jack Russell terrier	AD	+	<i>D. pteronyssinus</i>
AD10	15	M	Cross-breed	AD	–	<i>Aspergillus fumigatus</i>
AD11	10	M	Labrador retriever	AD	–	<i>P. americana</i>
AD12	1	M	Thai ridgeback	AD	–	<i>Culex pipiens</i>
AD13	6	F	Cross-breed	AD	–	<i>P. americana</i>
AD14	2	M	West Highland white terrier	AD	–	Mixed ants
AD15	7	F	Poodle	AD	–	<i>C. pipiens</i>
AD16	5	M	Pug	AD	–	<i>P. americana</i>

AD atopic dermatitis, Der f *Dermatophagoides farinae*, IDT intradermal skin test.

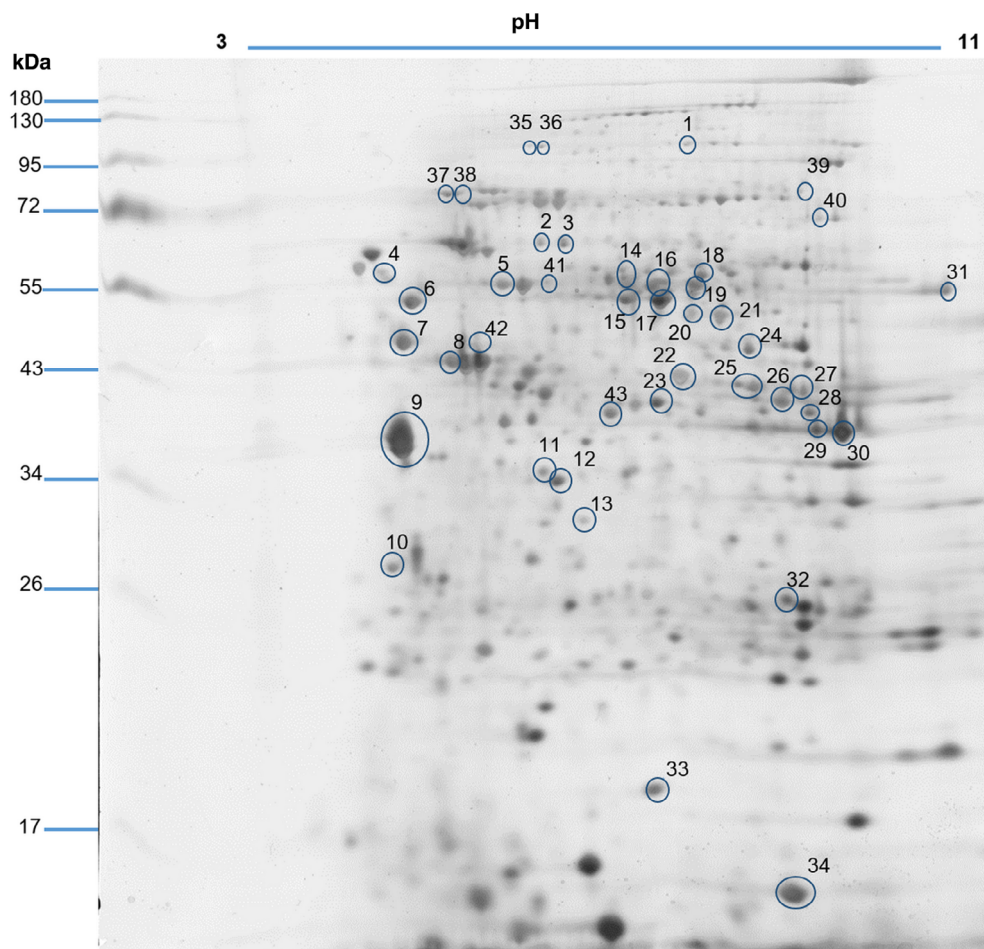


Figure 1. Representative 2D electrophoresis (2DE)-gel of *Dermatophagoides farinae* extracts.
pI isoelectric point; ○ immunoglobulin (Ig)E-reactive spots that were found among the study population.

components in this group. In the AD-IDT to Der f group, after analysis, all IgE-binding components were found to be the same as those found in the AD + IDT to Der f group, except for one hypothetical protein [spot 30; glyceraldehyde 3-phosphate dehydrogenase (G3PDH)], which was identified as a major IgE-binding component for this group only.

Discussion

This study of Thai dogs with AD identifies a set of specific allergens in Der f extracts to which their serum IgE binds. The crude Der f extracts contained both specific allergens related to AD, and a group of nonallergenic components: this emphasises the potential to use differential protein markers in diagnosis. Previously, high serum levels of allergen-total IgE and allergen-specific IgE as determined by ELISA were not to be exclusively with canine AD.^{21,22} However, by using their 2DE pattern, the type and number of IgE-binding components showed a distinguishable pattern between healthy and atopic dogs in this study. In healthy dogs, common allergens binding to IgE were found in very low numbers. By contrast, a higher number of IgE-binding components were found in Der f-allergic atopic dogs and in non-Der f-allergic atopic

dogs, especially among high molecular weight proteins. We suggest that certain IgE-binding components may still act as common antigens in healthy dogs via exposure to other natural antigens. Interestingly, using the crude Der f extract preparation that contains all of the IgE-binding components probably reduces specificity yet might increase sensitivity to serological detection because of the nonspecific allergens that are present.

Based on the recognition profile in atopic dogs, 25 components were identified. Unfortunately, eight hypothetical proteins were found with doubtful characterisations owing to the limits of the available orthologous proteins in the database. Similar to the situation with AD in humans, atopic dogs showed polysensitisation to a broad range of components, especially in the AD + IDT to Der f group.²³ Der f Alt a 10, EFl- α , gelsolin-like allergen Der f 16, and Der f 2 showed an outstanding percentage of recognition (>50%) in the AD + IDT to Der f group compared to the AD - IDT to Der f group. In humans, Der f Alt a 10 and gelsolin-like allergen Der f 16 have been reported as a new class of allergens,^{23,24} and this is the first time that these have been reported in atopic dogs. The Der f Alt a 10 showed a high homology to aldehyde dehydrogenase of *A. alternata* named "Alt a 10". To date, the allergenicities of Der f Alt a 10 and gelsolin-like allergen Der f 16 in both humans and dogs have not been elucidated.



Figure 2. Representative results of 2D electrophoresis (2DE)-immunoglobulin (IgE) immunoblotting in healthy and atopic dogs. The crude extracts of *Dermatophagoides farinae* (Der f) were subjected to 2DE and probed with serum samples. Probing with: (a) pooled sample from healthy dogs (H1-H5); (b) sample from Der f allergy-atopic dog AD8; and (c) sample from non-Der f allergy-atopic dog AD13.

In our study, the protein EFI- α found in the AD + IDT group was reported previously as a potential allergen from the American cockroach (*Periplaneta americana*),²⁵ yet their allergenicity has not been confirmed either in atopic humans or atopic dogs. To prove the importance of this allergen in atopic dogs, more evidence from skin testing with purified EFI- α or from studies into the prevalence of IgE-binding EFI- α is required. Additionally, the Der f 2 protein has been reported as a major allergen component in human patients,^{23,26} and we found this consistently in our atopic dogs. However, it is important to note that differences associated with different geographical regions might lead to unique major protein components.^{15,18} In this study of Thai atopic dogs, Der f 2 appeared to act as a major allergen bound to IgE, and this also has been reported in atopic dogs from Japan.¹⁵ Therefore, further studies are justified to investigate monotherapy of canine AD using pullulan-conjugated Der f 2 allergen-specific immunotherapy.¹²

The IgE of four of seven non-Der f-allergic atopic dogs that were sensitised to American cockroach

allergen bound to arginine kinase. It has been speculated that arginine kinase may act as cross-reactive invertebrate components found in canine and human allergies.^{27,28} In humans, arginine kinase induces helper T (Th)2 cell differentiation that results in histamine release.^{29,30} However, the nonspecific allergens alpha-enolase and serine protease jeopardise epithelial barrier function.^{31–33} Attention should be paid to cross-reactive invertebrate components in canine allergic patients, in term of pathogenesis. We suggest that the nonspecific proteins arginine kinase, serine protease and two types of hypothetical proteins (cytochrome c oxidase subunit VIb and microcephalin) that occur in crude Der f preparations should be considered as possibly reducing the specificity of ELISA detection, especially for IgE-based serology testing.

In accordance with findings in asthmatic human patients,^{34,35} this study supported that Der f 28 (heat shock protein 70 kDa) was a new allergen component related to Der f F sensitisation in 88.9% (eight of nine) of atopic dogs. Accordingly, Der f 28 is an interesting

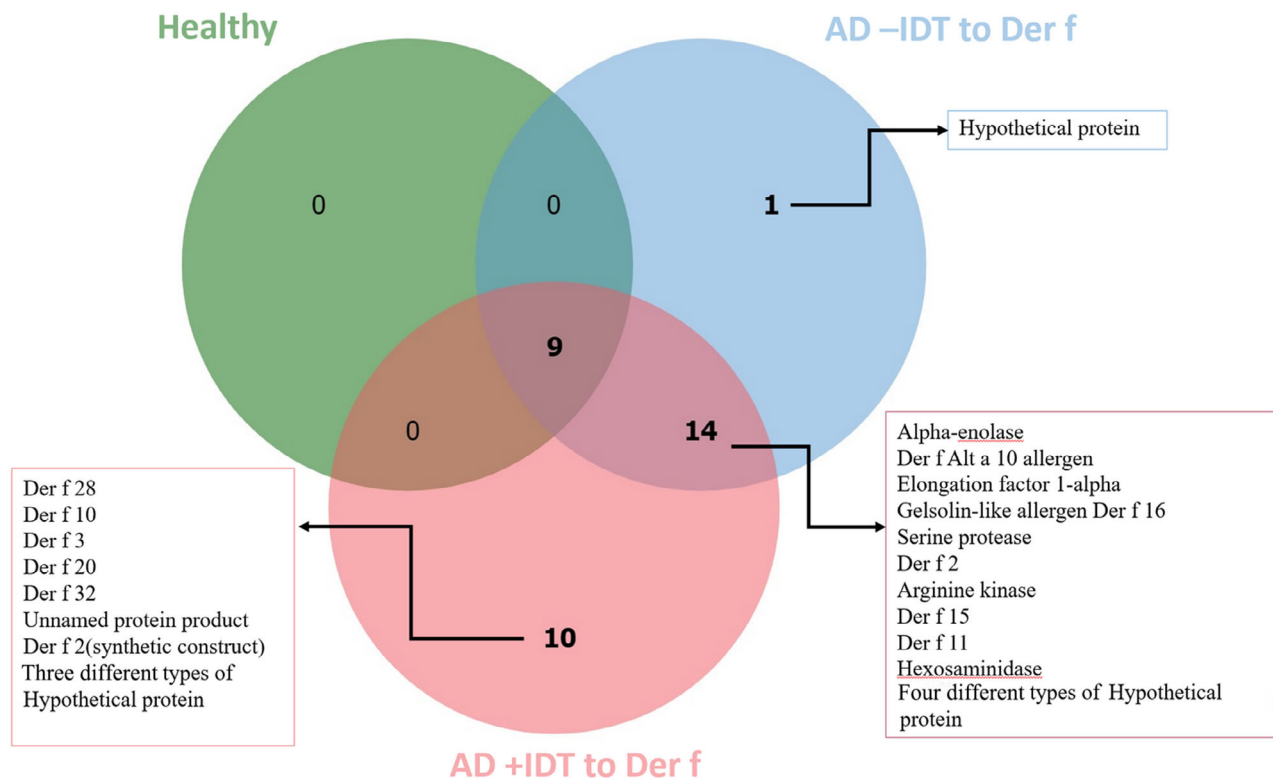


Figure 3. Results of number and types of immunoglobulin (IgE) recognised components to *Dermatophagoides farinae* (Der f) extracts in each group of dogs.

AD atopic dogs; $\pm$ IDT positive or negative intradermal testing skin reaction.

component to investigate for future use in component-resolved diagnostics (CRD) and CRIT.

In our study, multiple IgE-binding components were found in atopic dogs, including Der f 3, Der f 10, Der f 20 and Der f 32. Interestingly, Der f 3 and Der f 20 contain serine protease and arginine kinase-like domains in their structures, respectively, and IgE bound by Der f 3 and Der f 20 was found only in the AD + IDT to Der f group. These proteins may represent clinical component markers of Der f allergy, without reaction of cross-reactive invertebrate components. However, their immunopathology needs to be confirmed in atopic dogs. In the AD + IDT to Der f group, Der f 10 was found as a minor IgE-binding component (44% recognition; four of nine): this finding was in accordance with the results of a previous study (30% recognition; five of 17).¹⁶ In humans, Der f 10 was identified as tropomyosin and suspected as being a cross-reactive component in allergic reactions to crustacean shellfish, cockroach and HDMs.³⁶ Overall, these three components (Der f 3, Der f 10, Der f 20) were specific allergenic proteins related to Der f allergy in our atopic dogs, so that these are not specific to human patients. In addition, Der f 32 was one of the minor components in Der f-allergic atopic dogs as well as in humans.²³ However, the current identification of Der f 32 is only one of a few reports in atopic dogs, and intensive investigation will be necessary to determine its usefulness for clinical application.

Unexpectedly, we found that Der f 15 and an unnamed protein (spot 28) that were identified as chitinase were minor allergens (<50% recognition), contrary to previous

studies in North America and Europe where Der f 15 was recorded as a major Der f component.¹⁶ Previously, a Der f 15-like 98 kDa protein was recognised as a minor allergen in atopic dogs in Japan.¹⁵ Besides geographical variations, differences in source and preparation of allergen extracts could be a reason for protein size differences and frequency of recognition in different studies.^{14–16,37} In our study, Der f 15 was identified at 63.2 kDa, smaller than in the previous report from Japan.¹⁵ Although this may be due to protein deglycosylation,¹⁶ the glycosylation variant of Der f 15 might still maintain the IgE-binding recognition.¹⁶ To date, whether glycosylation of Der f 15 affects IgE recognition has not been determined in either humans or dogs.

In 2DE each protein is shown as one spot, based on separation by charge and mass, whereas in 1DE one band may contain multiple proteins. Proteins with a size >170 kDa and/or pH out of range (pH 3–11), or that have a high hydrophobicity cannot be isolated by 2DE. Unfortunately, this may be a reason why the novel allergen “Zen-1” (150–250 kDa) was not found in our study.¹⁸

Interestingly, samples from the AD – IDT to Der f group were strongly associated with G3PDH, at $\leq 71.4\%$. G3PDH has been reported as an allergen component of the American cockroach in the website of Allergen Nomenclature, called Per a 13. Two of three atopic dogs that were allergic to American cockroach had an IgE recognition to G3PDH of Der f in our study. However, information about this allergen is limited, so further investigation is required to clarify the significance of this finding.

Table 2. Immunoglobulin (Ig)E-recognised *Dermatophagoides farinae* (Der f) components in the two groups AD + IDT to Der f and AD – IDT to Der f (AD atopic dermatitis, IDT intradermal testing)

Spot no.	Allergen/ protein name	AD + IDT to Der f* no.									Frequency	%	AD – IDT to Der f† no.								Frequency	%
		1	2	3	4	5	6	7	8	9			10	11	12	13	14	15	16			
15,17,19,20	Alpha-enolase	+	+	+	+	+	+	+	+	+	9	100	+	+	–	+	+	+	+	6	85.7	
5	Der f Alt a 10 allergen	+	+	+	+	–	+	+	+	+	8	88.9	–	+	+	+	–	–	–	3	42.9	
7,31	Elongation factor 1- alpha	+	+	+	+	+	+	+	+	–	8	88.9	+	–	–	–	–	–	+	2	28.6	
29, 32	Der f 28	+	+	+	+	+	+	+	+	–	8	88.9	–	–	–	–	–	–	–	0	0	
14,16	Gelsolin-like allergen Der f 16	+	–	–	+	+	+	+	+	+	7	77.8	–	–	–	+	–	–	–	1	14.3	
18,33	Serine protease	–	–	+	+	+	+	+	+	+	7	77.8	+	+	–	+	+	–	–	4	57.1	
34	Der f 2	+	+	–	+	+	+	+	+	–	7	77.8	–	–	–	–	–	+	–	1	14.3	
22	Hypothetical protein	–	–	–	+	+	+	+	+	+	6	66.7	+	+	–	+	+	+	+	6	85.7	
6	Hypothetical protein	+	–	+	+	+	–	–	+	–	5	55.6	+	+	–	–	+	–	+	4	57.1	
25,27	Arginine kinase	+	+	–	–	–	+	+	+	–	5	55.6	+	–	+	+	–	–	+	4	57.1	
9,10	Der f 10	–	–	+	+	+	+	–	–	–	4	44.4	–	–	–	–	–	–	–	0	0	
13	Der f 3	–	–	–	+	–	+	+	+	–	4	44.4	–	–	–	–	–	–	–	0	0	
21	Hypothetical protein	–	+	–	–	+	+	+	–	–	4	44.4	–	–	–	–	–	–	–	0	0	
23	Hypothetical protein	–	–	–	+	–	–	+	+	+	4	44.4	–	+	–	–	+	–	–	2	28.6	
26	Der f 20	–	–	–	–	+	+	+	+	–	4	44.4	–	–	–	–	–	–	–	0	0	
2	Der f 15	–	–	–	+	–	+	–	+	–	3	33.3	–	+	+	+	–	–	–	3	42.9	
3	Hypothetical protein	–	–	–	+	–	+	–	+	–	3	33.3	–	+	+	+	–	–	–	3	42.9	
4	Der f 11	+	+	+	–	–	–	–	–	–	3	33.3	–	+	–	–	–	–	–	1	14.3	
8	Hexosaminidase	–	+	–	+	–	+	–	–	–	3	33.3	–	–	+	+	–	–	–	2	28.6	
12	Der f 32	–	–	–	+	–	–	+	+	–	3	33.3	–	–	–	–	–	–	–	0	0	
28	Unnamed protein product	–	+	–	–	–	+	–	+	–	3	33.3	–	–	–	–	–	–	–	0	0	
11	Hypothetical protein	–	–	–	+	–	–	+	–	–	2	22.2	–	–	–	–	–	–	–	0	0	
1	Der f 2 (synthetic construct)	–	–	+	–	–	–	–	–	–	1	11.1	–	–	–	–	–	–	–	0	0	
24	Hypothetical protein	–	–	–	+	–	–	–	–	–	1	11.1	–	–	–	–	–	–	–	0	0	
30	Hypothetical protein	–	–	–	–	–	–	–	–	–	0	0	+	+	–	+	+	+	–	5	71.4	

*Atopic dogs that had positive skin reaction to *D. farinae*.†Atopic dogs that had negative skin reaction to *D. farinae*.**Table 3.** Accession number and protein function of each component recognised by immunoglobulin (Ig)E

Spot no.	GenBank no.	Allergen/protein name	Biological function/ identity/ conserved domain
15, 17, 19, 20	AHV90299	Alpha-enolase	Enolase
5	AAP35081	Der f Alt a 10 allergen	Aldehyde dehydrogenase
7, 31	ABW99067	Elongation factor 1- α	Elongation factor 1-alpha; Provisional
29, 32	AIO08848	Der f 28	Heat shock 70 kDa protein; Provisional
14, 16	AAM64112	Gelsolin-like allergen Der f 16	Gelsolin sub-domain 1- and 3-like domain
18, 33	AAN02510	Serine protease	Trypsin-like serine protease
13	AAA99805	Der f 3	Trypsin-like serine protease
34	ABG35122	Der f 2	NPC2 family
1	CAA00161	Der f 2 [synthetic construct]	NPC2 family
22	EFY86341	Hypothetical protein	Microcephalin and similar proteins
11	XP_001868513	Hypothetical protein	Microneme/rhoptry antigen; Provisional
6	KJZ80397	Hypothetical protein	Cytochrome c oxidase subunit VIb
25, 27	AAP57094	Arginine kinase	Arginine kinase
26	AIO08850	Der f 20	Arginine kinase
9, 10	BAA04557	Der f 10	Tropomyosin
2	AAD52672	Der f 15	Chitinases
28	CAD35266	Unnamed protein product	Chitinases
4	EFP06016	Der f 11	Paramyosin
8	AIO08858	Hexosaminidase	Beta-N-acetylhexosaminidases
12	AIO08849	Der f 32	Inorganic pyrophosphatase
3	EZA54080	Hypothetical protein	Unknown
21	EGX95844	Hypothetical protein	Unknown
23	XP_009014800	Hypothetical protein	Isocitrate/isopropylmalate dehydrogenase
24	EPY82997	Hypothetical protein	Intermediate filament protein
30	ACG63665	Hypothetical protein	Glyceraldehyde-3-phosphate dehydrogenase

Der f *Dermatophagoides farinae*.

Our study contained several limitations regarding the clinical significance of the existence of multiple IgE-recognised Der f components in dogs with AD, and in bridging the disparity between findings in humans and dogs. In future studies we intend to increase the sample size and categorise groups of dogs according to their clinical severity and pattern of allergen sensitisation. Moreover, the next study will examine cross-reactive components among invertebrates and between species of HDM.

Conclusions

Multiple IgE-binding components of crude Der f extracted proteins were demonstrated and identified among Thai atopic and healthy dogs. Five allergens including Der f Alt a 10 allergen, EF1- α , gelsolin-like allergen Der f 16, Der f 28 and Der f 2 were identified as the major IgE-binding components that were specific to Der f allergy, while Der f 3, Der f 10, Der f 20 and Der f 32 were recognised as minor relevant allergens. Cross-reactive invertebrate components were suspected in cases of IgE binding to alpha-enolase, serine protease and arginine kinase. This study identifies the relevant allergen component markers that can be used to enhance specificity for diagnosis and treatment of atopic dogs in Thailand.

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Résumé

Contexte – *Dermatophagoides farinae* (Der f) est un allergène fréquent chez le chien atopiques (AD). Cependant, les composants pertinents de Der f nécessitent plus d'études.

Objectifs – Nous voulons fournir des données sur les immunoglobulines (Ig)-E spécifiques des composants de Der f pour applications diagnostiques et thérapeutiques.

Sujets – Les échantillons de serum ont été prélevés de cinq chiens sains, neufs atopiques allergiques à Der f et sept chiens atopiques non allergiques à Der f identifiés basés sur des tests intradermiques.

Méthodes – Nous explorons le profil d'extraits de Der f avec électrophorèse sur gel en 2D et immunoblotting IgE. Les composants se liant aux IgE des deux groupes de chiens atopiques ont été analysés par spectrométrie de masse.

Résultats – La majorité des chiens atopiques allergiques à Der f reconnaissent Der f *Alternaria alternata* allergen 10 (Der f Alt a 10), elongation factor 1- α (EF1- α), gelsolin-like allergène Der f 16, Der f 28 et Der f 2. Der f 3, Der f 10, Der f 20 et Der f 32 étaient reconnus comme allergènes mineurs. Alpha-énolase, serine protéase, arginine kinase et quelques protéines hypothétiques ont été reconnues dans les deux groupes de chiens atopiques. De façon inattendues, Der f 15 (chitinase) a été trouvé comme composant mineur.

Conclusions et importance clinique – De multiples allergènes se liant aux IgE de Der f ont été identifiés chez les chiens atopiques Thai. Nous proposons la série d'antigènes spécifiques qui se lient aux IgE, comprenant Der f Alt a 10, EF1- α , gelsolin-like Der f 16, Der f 28 et Der f 2, qui pourrait être utilisé pour de futurs diagnostics et plateformes d'immunothérapie.

Resumen

Introducción – *Dermatophagoides farinae* (Der f) es un alérgeno común en perros con dermatitis atópica (EA). Sin embargo, los componentes relevantes de Der f requieren más investigación.

Objetivos – Nuestro objetivo era proporcionar datos sobre los componentes específicos de Der f que se unen a inmunoglobulina (Ig)E para otras aplicaciones diagnósticas y terapéuticas.

Animales – se recogieron muestras de suero de cinco perros sanos, nueve perros atópicos alérgicos a Der f y siete atópicos no alérgicos a Der f identificados en base a una prueba cutánea intradérmica.

Métodos – exploramos los perfiles de los componentes de los extractos de Der f mediante electroforesis en gel 2D e inmunotransferencia de IgE. Los componentes de unión a IgE en ambos grupos de perros atópicos se analizaron mediante espectrometría de masas.

Resultados – la mayoría de los perros atópicos alérgicos a Der f reconocieron el alérgeno 10 de Der f *Alternaria alternata* (Der f Alt a 10), el factor de elongación 1- α (EF1- α), el alérgeno similar a la gelsolina Der f 16, Der f 28 y Der f 2. Der f 3, Der f 10, Der f 20 y Der f 32 fueron reconocidos como alérgenos menores. La alfa-enolasa, la serina proteasa, la arginina quinasa y algunas proteínas hipotéticas fueron componentes reconocidos en ambos grupos de perros atópicos. Inesperadamente, se encontró que Der f 15 (quitinasa) era un componente menor.

Conclusiones e importancia clínica – Se identificaron múltiples alérgenos de Der f que se unen a IgE en perros atópicos tailandeses. Proponemos que el conjunto de antígenos específicos que está unido por IgE, que comprende Der f Alt a 10, EF1- α , Der f 16 similar a gelsolina, Der f 28 y Der f 2, podría usarse para futuras plataformas de diagnóstico e inmunoterapia.

Zusammenfassung

Hintergrund – *Dermatophagoides farinae* (Der f) ist ein häufiges Allergen bei Hunden mit atopischer Dermatitis (AD). Nichtsdestotrotz sind weitere Untersuchungen in Bezug auf die relevanten Komponenten von Der f nötig.

Ziele – Unser Ziel war es, Daten über die Immunglobulin (Ig)E-bindenden spezifischen Komponenten von Der f für weitere diagnostische und therapeutische Anwendungen zu liefern.

Tiere – Es wurden Serumproben von fünf gesunden, neun Der f-allergischen atopischen und sieben nicht-Der f-allergischen atopischen Hunden, welche basierend auf einem Intradermaltest identifiziert worden waren, genommen.

Methoden – Wir untersuchten die Komponentenprofile der Der f Extrakte mittels 2D Elektrophorese und IgE Immunblotting. Es wurden die IgE-bindenden Komponenten beider Gruppen atopischer Hunde mittels Massenspektrometrie analysiert.

Ergebnisse – Die Mehrheit der Der f-allergischen atopischen Hunde erkannten Der f Alternaria alternata Allergen 10 (Der f Alt a 10), Elongation Factor 1-alpha (EF1- α), Gelsolin-like Allergen Der f 16, Der f 28 und Der f 2. Der f 3, Der f 10, Der f 20 und Der f 32 wurden als Minorallergene erkannt. Alpha-Enolase, Serinprotease, Argininkinase und einige hypothetische Proteine wurden in beiden Gruppen der atopischen Hunde erkannt. Unerwarteterweise wurde festgestellt, dass Der f 15 (Chitinase) eine Minorkomponente darstellt.

Schlussfolgerungen und klinische Bedeutung – Bei thailändischen atopischen Hunden wurden multiple IgE-bindende Allergene von Der f identifiziert. Wir schlagen vor, dass das spezifische Antigen Set, welches an IgE bindet und aus Der f Alt a 10, EF1- α , Gelsolin-like Der f 16, Der f 28 und Der f 2 besteht, in der Zukunft zur Diagnostik und Immuntherapie eingesetzt werden könnte.

要約

背景 – *Dermatophagoides farinae* (Der f)は、アトピー性皮膚炎(AD)の犬によく見られるアレルゲンである。しかし、Der fの関連成分については、さらなる調査が必要である。

目的 – 本研究の目的は、さらなる診断および治療応用のために、Der fの免疫グロブリン(Ig)E結合特異的成分に関するデータを提供することであった。

被験動物 – 5頭の健康犬、9頭のDer fアレルギー性アトピー犬、7頭の非Der fアレルギー性アトピー犬から血清サンプルを採取し、皮内試験に基づいて同定した。

方法 – 二次元ゲル電気泳動法およびIgEイムノブロットング法により、Der f抽出物の成分プロファイル調べた。また、両群のアトピー犬のIgE結合成分を質量分析法で解析した。

結果 – Der fアレルギー性アトピー犬の大多数は、Der f Alternaria alternata allergen 10 (Der f Alt a 10)、伸長因子1-a (EF1-a)、ゲルゾリン様アレルゲンDer f 16、Der f 28、Der f 2を認識し、Der f 3、Der f 10、Der f 20、Der f 32はマイナーアレルゲンとして認識した。a-エノラーゼ、セリンプロテアーゼ、アルギニンキナーゼおよびいくつかの仮説的タンパク質は、アトピー犬の両群で認識される成分であった。予期せぬことに、Der f 15 (キチナーゼ)はマイナーな成分であることがわかった。

結論と臨床的重要性 – タイのアトピー犬において、Der fの複数IgE結合アレルゲンが同定された。我々は、Der f Alt a 10、EF1-a、ゲルゾリン様Der f 16、Der f 28、Der f 2からなるIgEに結合する特異的な抗原セットが、将来の診断や免疫療法のプラットフォームに利用できることを提案する。

摘要

背景 – 粉尘螨(Der f)是特应性皮炎(AD)犬的常见过敏原。然而，Der f的相关组成部分需要进一步研究。

目的 – 我们旨在提供Der f免疫球蛋白(Ig)E结合特异性组分的数据，用于进一步的诊断和治疗应用。

动物 – 从根据皮内皮肤试验确定的5只健康、9只Der f过敏性特应性和7只非Der f过敏性特应性犬中采集血清样本。

方法 – 我们通过2D凝胶电泳和IgE免疫印迹探索了Der f提取物的组分谱。通过质谱法分析两组特应性犬的IgE结合组分。

结果 – 识别出Der f过敏性特应性犬的多数过敏原是Der f互隔交链孢霉10(Der f Alt a 10)、延长因子1- α (EF1- α)、凝溶胶样Der f 16、Der f 28和Der f 2。识别出少数过敏原是Der f 3、Der f 10、Der f 20和Der f 32。a-烯醇化酶、丝氨酸蛋白酶、精氨酸激酶和一些假定蛋白是两组特应性犬中公认的组分。出乎意料的是，Der f 15 (几丁质酶)被发现是一个少数组分。

结论和临床重要性 – 在泰国特应性犬体内，确定了Der f的多种IgE结合过敏原。我们建议与IgE结合的特异性抗原组，包括Der f Alt a 10、EF1- α 、凝溶胶样Der f 16、Der f 28和Der f 2，可用于未来的诊断和免疫治疗平台。

Resumo

Contexto – *Dermatophagoides farinae* (Der f) é um alérgeno comum em cães com dermatite atópica (DA). Entretanto, os componentes relevantes de Der f requerem mais investigação.

Objetivos – Nosso objetivo foi fornecer dados sobre os componentes específicos da ligação de imunoglobulina (Ig) E a Der f para posteriores aplicações diagnósticas e terapêuticas.

Animais – Amostras de soro foram coletadas de cinco cães saudáveis, nove atópicos alérgicos a Der f e sete atópicos não alérgicos a Der f identificados com base em um teste intradérmico.

Métodos – Exploramos os perfis de componentes dos extratos de Der f por meio de eletroforese em gel 2D e imunoblotting de IgE. Os componentes de ligação de IgE em ambos os grupos de cães atópicos foram analisados por espectrometria de massa.

Resultados – A maioria dos cães atópicos alérgicos a Der f reconheceu o alérgeno 10 *Alternaria alternata* do Der f (Der f Alt a 10), fator de alongamento 1-alfa (EF1- α), alérgeno semelhante à gelsolina Der f 16, Der f 28 e Der f 2. Der f 3, Der f 10, Der f 20 e Der f 32 foram reconhecidos como alérgenos menores. Alfa-enolase, serina protease, arginina quinase e algumas proteínas hipotéticas foram componentes reconhecidos em ambos os grupos de cães atópicos. Inesperadamente, Der f 15 (quitinase) foi considerado um componente secundário.

Conclusões e importância clínica – Múltiplos alérgenos de ligação de IgE de Der f foram identificados em cães atópicos tailandeses. Propomos que o conjunto de antígenos específicos que é ligado por IgE, compreendendo Der f Alt a 10, EF1- α , Der f 16 semelhante a gelsolina, Der f 28 e Der f 2, poderia ser usado para plataformas futuras de diagnóstico e imunoterapia.