

ORIGINAL PAPER

The chitinase allergens Der p 15 and Der p 18 from *Dermatophagoides pteronyssinus*

S. E. O'Neil*, T. K. Heinrich*, B. J. Hales*, L. A. Hazell*, D. C. Holt†, K. Fischer‡ and W. R. Thomas*

*Division of Molecular Biotechnology, Telethon Institute for Child Health Research, Centre for Child Health Research, University of Western Australia, Subiaco, WA, Australia, †Menzies School of Health Research, Casuarina, NT, USA, and ‡Queensland Institute of Medical Research, Malaria and Scabies Laboratory, PO Royal Brisbane Hospital, Brisbane, QLD, Australia

Clinical and Experimental Allergy

Summary

Background House dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* cause allergic disease in humans as well as in dogs. In geographical regions where the two mite species coexist, they both elicit specific immunoglobulin (Ig E) responses in humans whereas dogs preferentially react to *D. farinae* extracts. In dogs the main IgE binding is directed to the *D. farinae* chitinase allergens Der f 15 and Der f 18 and not to the groups 1 and 2 allergens as found for humans. Although the IgE response of humans to Der f 18 has been investigated there is no report on Der f 15-specific IgE in humans.

Objective This study aimed to characterize the chitinase allergens Der p 15 and Der p 18 of *D. pteronyssinus* and to find out whether they are important allergens for humans.

Methods cDNA was cloned by a polymerase chain reaction strategy from *D. pteronyssinus* libraries using primers based on conserved chitinase sequences. IgE binding to the recombinant polypeptides was measured by immunosorbent assay. Mice were immunized with the polypeptides and cross-reactivity examined.

Results Two variants of Der p 15 were isolated, encoding mature proteins of 58.8 and 61.4 kDa. The amino acid sequences had 90% identity to Der f 15. The cDNA for Der p 18 encoded a mature protein of 49.2 kDa with 88% sequence identity to Der f 18. Der p 15-specific IgE was detected in 70% and Der p 18-specific IgE in 63% of a panel of 27 human allergic sera.

Conclusions The *D. pteronyssinus* chitinases Der p 15 and Der p 18 show a high frequency of binding to IgE in allergic human sera. They are therefore potentially important allergens for humans as well as dogs.

Keywords allergen, chitinase, Der p 15, Der p 18, house dust mite, IgE binding

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Correspondence:

Tatjana Heinrich, Telethon Institute for Child Health Research, 100 Roberts Road Subiaco, WA 6008, Australia.
E-mail: tatjanah@icr.uwa.edu.au

Introduction

Dermatophagoides house dust mites (HDMs) are the most important source of indoor allergens associated with human asthma [1]. They also produce important allergens associated with atopic dermatitis in dogs as shown by intradermal skin tests with whole mite extracts [2–4]. In contrast to humans, dogs do not react strongly to the major human group 1 and 2 allergens but rather to high molecular weight proteins [5]. The high molecular weight chitinases Der f 15 [6] and Der f 18 [7] found in extracts of *Dermatophagoides farinae* have been identified as major dog allergens.

Chitinases (E.C.3.2.1.14) catalyse the hydrolysis of *N*-acetyl-D-glucosamine 1,4- β -linkages in chitin polymers.

They are essential for the arthropod life cycle by aiding the digestion of the exoskeleton during moulting. Chitinases are also important for arthropod gastrointestinal epithelia, where they are required for the maintenance of the tissue and the digestion of chitin-containing nutrients, which for mites, include chitin-coated dung balls.

The superior allergenicity of chitinases in canine allergy raises some interesting questions. Dogs must be exposed to these proteins in high enough quantities to induce sensitivity and allergic disease so it would be expected that humans sharing the same living environment would either become sensitized or alternatively develop protective, regulatory responses. Human responses to chitinase allergens have received little attention except for the study of Weber et al. [7], 2003. This study

reported that Der f 18 bound immunoglobulin (Ig) E from the sera of HDM allergic patients at a lower frequency (54%) than the groups 1 and 2 allergens. So far, there has been no report on Der f 15-specific IgE binding for humans but extrapolating from its higher frequency of IgE binding for dogs, it could be even more allergenic for humans than Der f 18. The human sera used by Weber *et al.* to study Der f 18 were obtained from allergic subjects in a region (Everett, WA, USA) where the most abundant mite is *Dermatophagoides pteronyssinus* [8, 9] and so the allergenicity of Der f 18 would be an underestimate. The studies to date have not examined *D. pteronyssinus*, which is the most prevalent pyroglyphid mite in all but the driest of climatic regions. The aim of this study was to identify the groups 15 and 18 allergens from *D. pteronyssinus* and to investigate their importance as human allergens.

Methods

cDNA and cDNA library

Live *D. pteronyssinus* were obtained from CSL-Ltd (Melbourne, Australia). Total RNA was extracted from whole mite bodies and converted into cDNA [10]. For direct PCR amplification, the cDNA was diluted 1 : 10 in 10 mM Tris/Cl, pH 8.5. The same cDNA was used to construct a cDNA library in Lambda Zap[®] Express (Stratagene, La Jolla, CA, USA) using the Zap[®] Express cDNA Synthesis and Cloning kit (Stratagene). Small volumes of glycerol stock of the library were diluted 1 : 10 in 10 mM Tris/Cl, pH 8.5 and used as template for PCR reactions.

DNA sequence analysis

DNA was sequenced by Sanger dideoxy chain termination using the Big Dye Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, CA, USA). The sequence was resolved on an ABI Prism 3730 48 capillary sequencer (Perkin Elmer). The sequences were analysed using SeqEd vs. 1.03 and MacVector software.

Cloning of cDNA encoding Der p 15 and Der p 18

A 1274 bp internal fragment of Der p 15 was isolated by PCR amplification of diluted *D. pteronyssinus* cDNA with primers to conserved chitinase motifs present in the Der f 15 gene. Sequencing of the internal fragment allowed synthesis of primers specific to Der p 15 which were used in conjunction with vector primers to amplify the missing regions of Der p 15 from a Lambda Zap[®] Express *D. pteronyssinus* cDNA library. Dilutions of library glycerol stocks served as polymerase chain reaction (PCR) template. The full-length Der p 15 cDNA was amplified with specific primers using high-fidelity *Pfu* DNA poly-

merase (Promega, Madison, WI, USA), A-tailed and cloned into the sequencing vector pGemT[™] (Promega). Sequencing revealed two highly homologous Der p 15 variants of 1677 and 1599 bp. The shorter variant is referred to as Der p 15 s.

A 557 bp internal fragment of Der p 18 was isolated by PCR amplification of diluted *D. pteronyssinus* cDNA with primers specific to conserved chitinase motifs unique for the Der f 18 gene. The full-length 1389 bp Der p 18 cDNA was obtained by the same strategy as outlined for Der p 15.

Human serum and plasma samples

Blood was obtained from 27 HDM allergic and four non-allergic people. The allergic subjects had doctor-diagnosed allergy or reported symptoms of asthma and/or rhinitis in a questionnaire. HDM allergy was corroborated by Pharmacia CAP[™] assay (performed by the Immunology Department at Princess Margaret Hospital; Subiaco, WA, Australia). All allergic sera had CAP scores of ≥ 2 equivalent to HDM-specific IgE ≥ 0.7 kU/L. The four non-allergic sera had HDM-CAP scores of zero. One of the control sera showed a CAP score of 5 to the major cat allergen Fel d 1. Serum was harvested within 2 h of blood collection and stored at -80°C ; in some cases where blood cells were harvested for other studies, plasma was collected. The Princess Margaret Hospital Human Ethics Committee approved the procedures in the study. All participants gave written consent to participate in the study.

Construction of Der p 15 and Der p 18 encoding expression vectors

The mature coding region of Der p 15 was amplified by high-fidelity *Pfu* DNA polymerase (Promega) with specific primers carrying restriction sites for *Bam*HI and *Hind*III. The restricted and purified PCR fragment was inserted into a *Bam*HI and *Hind*III digested expression vector pQE-80L (Qiagen Inc., Valencia, CA, USA), which adds an N-terminal hexa-His tag to the recombinant protein. The recombinant expression vector was cloned into *Escherichia coli* Top10 (Invitrogen, Groningen, Netherlands). The same strategy was used to generate a Der p 15 s and a Der p 18 encoding expression vector.

Expression and purification of recombinant Der p 15 and Der p 18

For expression of recombinant protein, Der p 15, Der p 15 s and Der p 18 encoding expression plasmids were transformed into *E. coli* BL21 Star (DE3) pLysS (Invitrogen). Both constructs were grown at 30°C in LB broth containing 100 $\mu\text{g/mL}$ of ampicillin, 34 $\mu\text{g/mL}$ chloramphenicol and 2% (w/v) glucose to an optical density (OD

600 nm) of 0.5. After induction with 1 mM isopropyl β -D-thiogalactopyranoside (IPTG), the culture was grown for a further 2 h before cells were spun down for 10 min at 4250 g at 4 °C. The cell pellet was stored at –20 °C for at least 4 h and thawed on ice to aid lysis. The pellet was resuspended in 2 mL of lysis buffer per gram of wet weight cells (50 mM NaH_2PO_4 pH 8.0, 600 mM NaCl, 20 mM imidazole), sonicated on ice and clarified by centrifugation for 30 min at 48 000 g at 4 °C.

To extract insoluble Der p 15 and Der p 18 contained in the inclusion bodies, the pellet was resuspended in Bacterial Protein Extraction Reagent (B-PER diluted 1:10; Pierce, Rockford, IL, USA), centrifuged for 15 min at 27 000 g at 4 °C and the supernatant discarded. After repeating two additional washes, the pellet was dissolved in 5 mL of 6 M guanidine hydrochloride pH 8.0 per gram of wet weight cells and incubated with vigorous shaking for 30 min at 37 °C. The supernatant was clarified by centrifugation for 18 h at 220 000 g, 4 °C, adjusted to 10 mM DTT and incubated at RT for 60 min. After protein quantification, protein was diluted to 50 $\mu\text{g}/\text{mL}$ in refolding buffer (5 mM reduced glutathione, 0.5 mM oxidized glutathione, 0.4 mM L-arginine, 150 mM NaCl, 20 mM Tris/Cl pH 8.5, 1 mM EDTA pH 8.0) containing 2 M urea. Refolding was achieved by five slow dialysis steps (no stirring) with equal amounts of refolding buffer without urea.

The protein solutions were dialysed into lysis buffer and purified by Ni NTA purification (Qiagen) under native conditions according to instructions of manufacturer. The recombinant allergens underwent further purification by gel filtration on HiPrepTM SephacrylTM S-200 HR (Amersham Biosciences, Uppsala, Sweden) in 20 mM Tris/Cl, pH 8.0, 500 mM NaCl.

Human-specific immunoglobulin E antibody assays

IgE binding was detected by antibody binding assay using time-resolved fluorescence (TRF) in the dissociation-enhanced lanthanide fluorescence immunosorbent assay for signal development (DELFIATM, Wallac, Turku, Finland). In this assay antibody binding is linked to the formation of a lanthanide chelate, which has different fluorescent properties than the unchelated form. This allows highly sensitive measurements over a wide range of concentrations with very little background interference. Purified allergens rDer p 2 [11], rDer p 15, rDer p 15s and rDer p 18 were coated at 5 $\mu\text{g}/\text{well}$ in $\text{Na}_2\text{CO}_3/\text{NaHCO}_3$ (pH 9.6) onto the microtitre plate (MaxiSorpTM, Nunc, Denmark) and incubated overnight at 4 °C. The plates were washed five times with wash buffer (Tris-HCl pH 7, 0.05% Tween 20) before blocking with 0.5% bovine serum albumin (in Tris-HCl, 0.05% Tween 20 buffer). After washing five times with wash buffer, twofold dilutions of the allergic and non-allergic plasma or serum samples were added to the

desired wells and incubated overnight at 4 °C. On the following day, the wells were washed five times with wash buffer and incubated with 100 μL of biotinylated mouse anti-human IgE mAb (BD Pharmingen, San Diego, CA, USA) diluted 1:1000 in assay buffer (Wallac) and incubated for 1 h at room temperature with shaking. After washing five times with wash buffer, the plate was incubated with 100 μL of Eu-streptavidin (Wallac) diluted 1:1000 in assay buffer for 30 min. After washing eight times with wash buffer, 100 μL of DELFIATM enhancement solution (Wallac) was added and incubated for at least 5 min. The fluorescence signals were measured on the Victor2 multilabel counter (Wallac) using a 405 nm filter.

Antibody and competition assays with polyclonal mouse sera

Groups of three C57BL/6 mice were injected with 100 μg of purified rDer p 15 or rDer p 18. After 5 weeks they were boosted with the same amount of protein. Serum was collected 10 days after boosting. The antibody assay was similar to the human-specific IgE immunoassay described above. The allergens were coated at a concentration of 1 $\mu\text{g}/\text{mL}$. After overnight incubation at 4 °C, 50 μL of polyclonal mouse sera (diluted 1:2000 in assay buffer, Wallac) and varying amounts of purified Der p 15 and Der p 18 (diluted in 50 μL of assay buffer) were applied and incubated for 2 h at room temperature under shaking. Biotinylated anti-mouse Ig from sheep (Amersham) diluted 1:10 000 was used as a second ligand and incubated for 1 h at room temperature under shaking. Development was carried out as described above.

Results

cDNA encoding Der p 15 and Der p 18

The Der p 15 cDNA revealed two variants, which differed by a deletion of 78 bp and a further base change, resulting in one amino acid substitution. The longer Der p 15 transcript (GenBank accession number DQ078740) was 1677 bp long, encoding a protein of 558 amino acids with an expected molecular mass of 61.4 kDa for the mature polypeptide. The shorter transcript was 1599 bp long, encoding a protein of 532 amino acids (GenBank accession number DQ078741) and is referred to as Der p 15s. The shorter polypeptide has a predicted molecular mass of 58.8 kDa. The deletion was found in a highly repetitive region (STP-rich region; Fig. 1) almost entirely made up of the amino acids serine (S), threonine (T) and proline (P). This region showed a strong resemblance to PEST-like regions (rich in amino acids P, E (glutamate), S and T), which have been described as one of the major features of insect chitinases [12]. Der p 15 and the long variant of Der p 15 had an STP-rich area of 83 and 86 amino acids, respectively, whereas Der p 15s had a deletion of 26 amino acids in this region. An alignment [13] of Der p

[illegible]

Fig. 1. Protein sequence alignment of Der p 15 (p15; GenBank accession number DQ078740), the short form of Der p 15 (p15s; GenBank accession number DQ078741) and Der f 15 (f15; GenBank accession number AF178772). Identical amino acids are indicated by dashed lines. The predicted leader sequence is shown in bold. The STP-rich region is highlighted in grey. Amino acid deletions in Der p 15s and Der f 15 are indicated by forward slashes (/). A potential *N*-glycosylation (NPT in position 132–134) site is double underlined. All other sequence motifs are surrounded by boxes and conserved amino acids are highlighted in grey. The conserved cysteine residues of the catalytic region are labelled C1–C4. Putative catalytic domains are labelled CD1 and CD2. The chitin-binding domain is labelled CBD.

15, Der p 15 s and Der f 15 (Fig. 1) showed 99.8% identity between the Der p 15 variants and 90% identity between Der p 15 and Der f 15 with an additional 5% of similar amino acids. To investigate the authenticity of the two Der p 15 isoforms, primers spanning the STP-rich region were designed. Two *D. pteronyssinus* cDNA libraries and one *D. pteronyssinus* genomic library underwent PCR with these primers and the expected long and short amplification products of the correct size were obtained from all three libraries (data not shown).

The Der p 18 cDNA (GenBank accession number DQ078739) encoded a polypeptide of 462 amino acids

(49.2 kDa expected molecular mass for the mature protein) and was identical in length to Der f 18. Both proteins had 88% identity and 60% of similar amino acids (Fig. 2).

Expression and purification of recombinant Der p 15 and Der p 18

N-terminal sequencing of the natural, mature proteins showed that Der f 15 and Der f 18 are secretory proteins with leader peptides of 20 and 25 amino acids, respectively [6, 7]. Considering the high sequence homology, the leader peptides of Der p 15, Der p 15 s and Der p 18 were

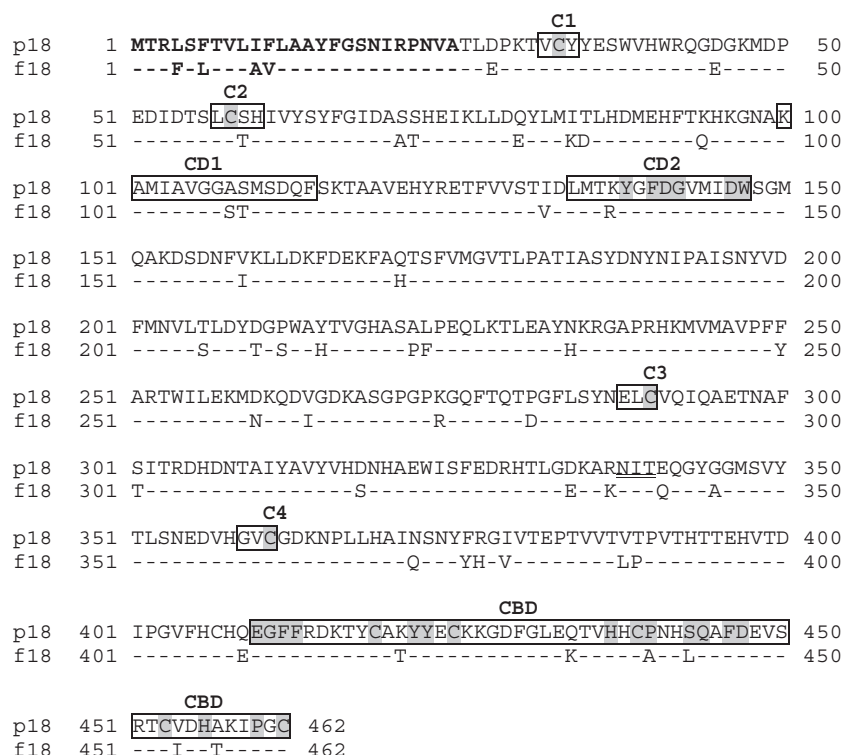


Fig. 2. Protein sequence alignment of Der p 18 (18; GenBank accession number DQ078739) and Der f 18 (f18; GenBank accession number AY093656). Identical amino acids are indicated by dashed lines. The predicted leader sequence is shown in bold. A potential N-glycosylation (NIT in position 338–340) site is double underlined. All other sequence motifs are surrounded by boxes and conserved amino acids are highlighted in grey. The conserved cysteine residues of the catalytic region are labelled C1–C4. Putative catalytic domains are labelled CD1 and CD2. The chitin-binding domain is labelled CBD.

assumed to have the same lengths as their *D. farinae* counterparts. The mature coding regions of Der p 15, Der p 15 s and Der p 18 were cloned into pQE-80L (Qiagen) for production of N-terminally hexa-His-tagged recombinant proteins. For all variations in culture temperature, induction times and medium composition examined, both polypeptides were expressed in inclusion bodies and were thus isolated as insoluble proteins and solubilized in non-denaturing buffer. The proteins were purified by Ni-affinity chromatography and gel filtration with a high-resolution Sephacryl S200 matrix. When examined by SDS-PAGE, the observed molecular mass of recombinant Der p 18 corresponded well with the predicted molecular mass of 51 kDa whereas the observed molecular masses of recombinant Der p 15 and Der p 15 s were approximately 8 kDa higher than the predicted values of 62 and 60 kDa. Neither N- nor O-glycosylation was expected to occur because the recombinant proteins were expressed in a bacterial host.

Immunoglobulin E binding of recombinant Der p 15 and Der p 18

A panel of 27 HDM allergic human sera and four control sera was tested by DELFIA for IgE binding to recombinant Der p 15, Der p 15 s, Der p 18 and Der p 2. The HDM-

specific IgE binding of the sera had been determined by Pharmacia CAPTM assay and was confirmed in this assay by strong IgE responses to the major *D. pteronyssinus* allergen Der p 2. Der p 15 and Der p 15 s bound IgE in 19 out of 27 subjects (70%). A cut-off of the mean + 2 standard deviations of the control sera was used. The control serum with high cat-specific IgE showed no IgE binding. Der p 18 bound IgE in 17 out of 27 (63%) of the sera. The TRF values for Der p 15 and Der p 18-specific IgE responses were usually lower than the responses for Der p 2 as shown by the correlation curves (Fig. 3). There were, however, seven sera with higher responses to both Der p 15 variants than to Der p 2 and nine sera with stronger responses to Der p 18 than to Der p 2.

There was no significant correlation ($r = 0.017$) between IgE reactivity to Der p 2 and Der p 15 (Fig. 3a) or Der p 18 ($r = 0.18$) (Fig. 3b). A strong correlation ($r = 0.93$) was shown for the two variants of Der p 15 (Fig. 3c). There was also a significant correlation ($r = 0.63$) between Der p 15 and Der p 18-specific IgE responses (Fig. 3d).

Cross-reactivity of rDer p 15 and rDer p 18

Polyclonal anti-Der p 15 and Der p 18 mouse sera were raised in C57BL/6 mice and tested by TRF immunosorbent

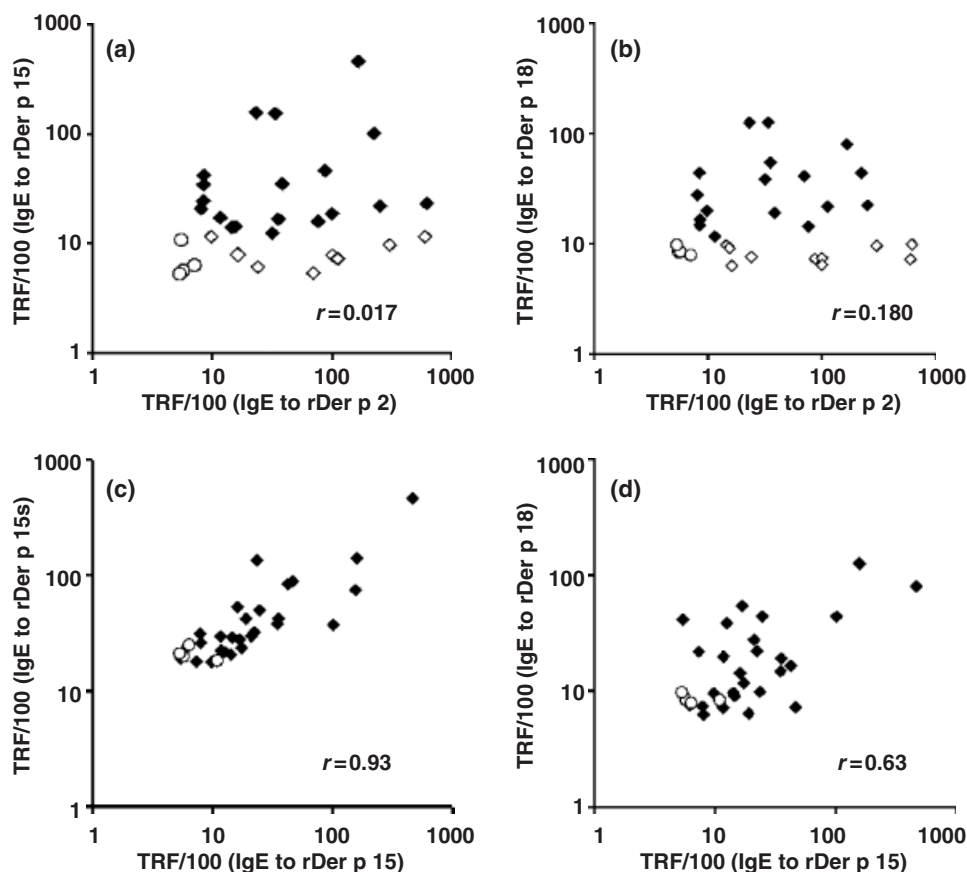


Fig. 3. Data obtained from human-specific immunoglobulin (Ig) E immuno assays. All time-resolved fluorescence (TRF) values were divided by 100 and graphed. Open circles represent non-allergic controls. Filled diamonds represent house dust mite allergic subjects reacting to Der p 2 as well as the tested allergen and open diamonds reacting to Der p 2 only. A: rDer p 15-specific IgE vs. rDer p 2 specific IgE response; B: rDer p 18-specific IgE vs. rDer p 2 specific IgE response; C: rDer p 15s-specific IgE vs. rDer p 15 specific IgE response; D: rDer p 18-specific IgE vs. rDer p 15 specific IgE response. The r -value represents correlation coefficient.

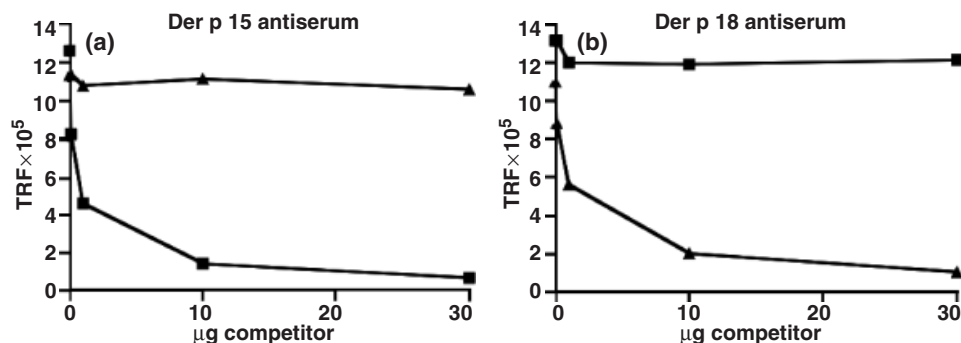


Fig. 4. Competition assays with anti-Der p 15 and anti-Der p 18 polyclonal mouse sera. All time-resolved fluorescence (TRF) values are graphed as $\text{TRF} \times 10^5$. Filled squares represent competition with Der p 15, filled triangles represent competition with Der p 18.

assay. The anti-Der p 15 serum bound to Der p 15, Der p 15s but not to Der p 18 (data not shown). The binding to Der p 15 was specifically inhibited by Der p 15 but not by Der p 18 (Fig. 4a). Equally, the anti-Der p 18 serum showed no cross-reaction to Der p 15 (data not shown) and the binding to Der p 18 was specifically inhibited only by Der p 18 (Fig. 4b).

Discussion

In this study, the chitinase allergens Der p 15 and Der p 18 from *D. pteronyssinus* have been characterized because of its global importance and because it is the dominant mite species in the study region [1]. Although the *D. farinae* chitinase allergens Der f 15 and Der f 18 have been

identified as the major IgE binding specificities of mite-allergic dogs [6, 7], only Der f 18 has been shown to bind to 54% of a panel of human HDM allergic sera. Because these sera were obtained from a company based in Everett, WA (USA), the values could be an underestimate because *D. pteronyssinus* has been reported to constitute 67% of the pyroglyphid mites in neighbouring Vancouver [8]. Allergens from *D. pteronyssinus* and *D. farinae* usually cross-react but large disparities in their IgE binding can be observed when studied quantitatively with purified allergens. Typically, the cross-reacting allergens have shown 10–30% of the IgE binding of the sensitizing specificity [14, 15].

Der p 18 had the same length as Der f 18 encoding a mature protein of 49 kDa with 88% amino acid identity and an additional 6% of similar amino acids. Similarly, Der p 15 had 90% identity to its Der f 15 counterpart. Unexpectedly, there were two 99.8% identical variants of the Der p 15 gene which differed in an internal domain almost entirely made up of the amino acids serine (S), threonine (T) and proline (P). This region resembled 'PEST-like' regions (rich in amino acids P, E (glutamate), S and T) [12], which are the target of extensive *O*-glycosylation and are regarded as one of the three basic domains found in insect chitinases. Der f 15 and the long form of Der p 15 contained an STP-rich sequence of 83 and 86 amino acids, whereas the short Der p 15 variant had a deletion of 26 amino acids in this region.

McCall et al. [6] isolated a pair of natural Der f 15 proteins with apparent molecular masses of 98 and 109 kDa. The proteins co-purified, their N-terminus was identical and they were recognized by the same antibodies. The authors showed that the proteins were heavily *O*-glycosylated leading to the discrepancy between the calculated (61.2 kDa) and apparent molecular mass and concluded that the 98 and 109 kDa proteins reflected glycosylation variants of the same protein. The finding here of two Der p 15 sequences encoding polypeptides differing in mass by 2.6 kDa would suggest that the 98 and 109 kDa Der f 15 proteins are variants and that the difference of 11 kDa in molecular mass is the result of an increased STP-rich region and the attendant increase in *O*-glycosylation.

The two variants of Der p 15 were isolated from a cDNA library, raising the question of whether or not they are the result of alternative splicing or products of different genes. PCR on two independent cDNA and one genomic *D. pteronyssinus* library with primers flanking the STP-rich region of Der p 15 resulted in a short and a long PCR amplification product from both types of library. The genomic amplification indicates that the different-length transcripts were products of two genes, which could be different alleles or loci, and did not result from alternative splicing.

Apart from the above-mentioned PEST-like domain, which is present in many chitinases but is not essential for function, all family 18 chitinases have an N-terminal glycosyl hydrolase catalytic region forming an extensive barrel structure. The active site is located in a groove of this structure and contains the highly conserved signature sequence YDFDGLDLWEYP [12]. The glutamate residue of this sequence is essential for the enzymatic activity. The third crucial domain of insect chitinases is a C-terminal chitin-binding region with four to six cysteine residues forming cystine bridges. Der p 15 possesses all three basic family 18 chitinase domains with a near perfect signature sequence in the catalytic site, an extensive PEST-like region and a type-2 chitin-binding domain [16]. Der p 18, on the other hand, has no PEST-like region and an imperfect family 18 catalytic domain with a truncated signature sequence lacking the essential glutamate residue. Der p 18 does, however, have a chitin-binding peritrophin-A domain, which is found in peritrophic matrix proteins of insects [16]. Recently, it has been shown that micro-organisms express several chitinases [17] and that efficient chitin degradation depends on the action of a non-catalytic chitinase-like protein, which binds to insoluble chitin, enhancing the activity of the catalytic chitinases. It is most likely that Der p 18 is such a non-catalytic chitinase.

Der p 15-specific IgE was detected in 70% and Der p 18-specific IgE in 63% of sera from a panel of 27 HDM allergic and four control human subjects. This is the first report of a group 15 reactivity in human sera, while the slightly higher prevalence of Der p 18-specific IgE is in good accordance with the Der f 18-specific IgE values found by Weber et al. [7]. The IgE binding to Der p 2 did not correlate significantly with the IgE binding to Der p 15 or Der p 18 and as shown by the correlation curves many of the sera with high binding to the chitinases had mid-low binding to Der p 2. The high IgE reactivity with polypeptides produced in *E. coli*, which has not been previously shown, demonstrates that the anti-chitinase activity is not directed to the carbohydrate moiety, a particular problem with the highly glycosylated group 15 allergen. The correlation in IgE binding between Der p 15 and Der p 18 was not anticipated because the allergens showed a very low 24% sequence identity and would not be expected to cross-react. Hyperimmune sera from mice to both *D. pteronyssinus* chitinases confirmed this by showing that there was no cross binding and no cross inhibition of anti-Der p 15 and Der p 18 sera. The correlation is more likely due to the finding that a subsection of allergic people is polysensitized, showing a broad sensitization profile to a number of mite allergens other than the groups 1 and 2 allergens [18, 19].

The human IgE binding prevalence of 60–70% to the chitinase allergens Der p 15 and Der p 18 is higher than that generally found for other pyroglyphid allergens

except for the groups 1 and 2 allergens [20–22]. Only anti-paramyosin [23, 24] and tropomyosin [25] responses in South East Asian countries have been reported to be higher and there is an as yet uncorroborated report for high IgE responses to a collagenolytic protease [26]. Another major specificity is the group 14 allergen M-177 whose degradation product shows high IgE binding in a Japanese test panel [27]. For tropomyosin the IgE reactivity appears to be a regional phenomenon perhaps associated with cross-reactivity, and paramyosin and the protease have not been extensively studied. The high IgE reactivity of group 15 and group 18 chitinases in humans along with the pre-eminence of these specificities in dog allergy points to the importance of studying these proteins both with respect to their allergenic potential and for studying how the responses to the different immunogenic proteins are regulated during sensitization.

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