

The isolation and characterization of a novel collagenolytic serine protease allergen (Der p 9) from the dust mite *Dermatophagoides pteronyssinus*

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Background: Dust mites have been shown to contain a serine protease distinct from the previously reported trypsin and chymotrypsin. The latter enzymes have been shown to be allergens, but the allergenic importance of the former is unknown.

Objective: This study was performed to isolate and characterize the novel mite serine protease and determine its allergenicity.

Methods: The mite serine protease was isolated from feces-enriched extracts of *Dermatophagoides pteronyssinus* by ion-exchange chromatography and affinity chromatography, and its physicochemical properties were determined. The allergenicity of the protease was assessed by using the RAST.

Results: The protease was enzymatically similar to chymotrypsin and cathepsin G-like enzymes from a variety of sources and was shown to cleave collagen. It had a molecular mass of 23,780 d. N-terminal sequence analysis (18 residues) indicated homology with the mite tryptic allergen, Der p 3, and the chymotryptic allergen, Der p 6. RAST analyses showed that the frequencies of reactivity to the novel allergen and to Der p 1, Der p 2, Der p 3, and Der p 6 were 92%, 97%, 100%, 97%, and 65%, respectively (n = 35). RAST inhibition studies showed some cross-reactivity between the protease and Der p 3 but not Der p 6.

Conclusions: A novel mite serine protease was isolated from *D. pteronyssinus* and found to be a major allergen. This allergen has been tentatively designated Der p 9. (*J Allergy Clin Immunol* 1996;98:739-47.)

Key words: Mites, allergy, proteases, allergens, characterization, asthma, trypsin, chymotrypsin

Studies from our laboratory and elsewhere have shown that some of the allergens from the clinically important dust mites are proteolytic enzymes.

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Abbreviations used

BAPNA:	N-benzoyl-arginine p-nitroanilide
MW:	Molecular weight
SA ₂ PFpNA:	Succinyl-ala-ala-pro-phe p-nitro-anilide
SA ₂ PLpNA:	Succinyl-ala-ala-pro-leu p-nitro-anilide
SA ₃ pNA:	Succinyl-ala-ala-ala p-nitroanilide
SBTI:	Soybean trypsin inhibitor
SDS-PAGE:	Sodium dodecylsulfate-polyacrylamide gel electrophoresis
SGM:	Spent growth medium

They include a cysteine protease (e.g., Der p 1)¹ and two serine proteases, namely, trypsin (e.g., Der p 3)² and chymotrypsin (e.g., Der p 6).³ The group 3 allergen, originally described by Heymann et al.,⁴

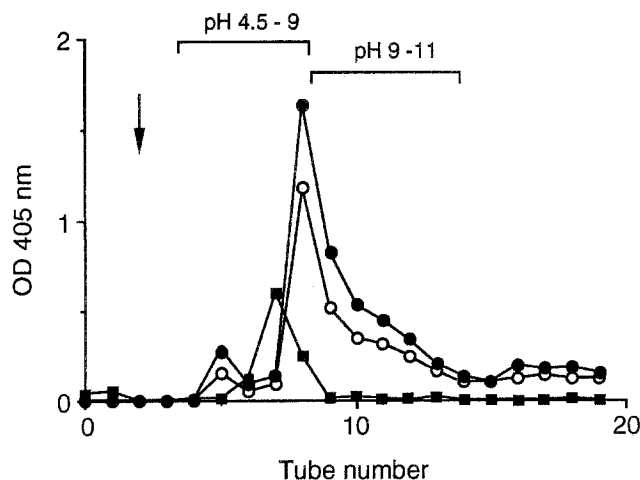


FIG. 1. Purification of Der p 9 by cation-exchange chromatography of SBTI affinity bound material. Bound material was eluted with distilled water adjusted to pH 11.0 with ammonia at point marked with the arrow. Protease activities were determined by using SA₂PLpNA (filled circles), SA₂PFpNA (open circles), and BAPNA (filled squares). Hydrolysis of synthetic substrates was measured at 405 nm. Two fractions eluting at the pH ranges indicated by horizontal bars were pooled for further analysis.

was isolated from feces-enriched extracts of *Der-matophagoides farinae* by gel filtration. Its apparent molecular weight (MW), isoelectric point, and N-terminal sequence were reported; and an equivalent protein was subsequently isolated from *D. pteronyssinus*.⁵ A comparison of the N-terminal sequences of Der f 3 and Der p 3 showed that they were about 70% and 50% identical with the serine proteases trypsin and chymotrypsin from vertebrate and invertebrate sources, respectively.^{2,5} Enzymatic studies confirmed that the group 3 allergens corresponded to trypsin,^{2,6} and recent data from cloning studies are consistent with these findings.⁷

The mite chymotrypsin allergens from *D. pteronyssinus* and *D. farinae* (Der p 6 and Der f 6, respectively) were originally isolated by Yasueda et al.² and have been shown to possess characteristics similar to those of the chymotrypsin previously reported elsewhere.⁸ The enzymes were shown to be acidic proteins with an apparent MW of 25,000 as determined by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions. The N-terminal sequences of Der p 6 and Der f 6 were shown to be about 70% identical, and both demonstrated about 60% identity with Der f 3. Yasueda et al.³ found that 41% of sera from patients allergic to mites recognized the group 6 chymotrypsins as determined by RAST. However, the frequency with which the group 3

trypsin allergens were recognized varied from 16% for the *D. farinae* allergen to 100% for the *D. pteronyssinus* allergen.²⁻⁴

The presence of a third serine protease in mite extracts prepared from *D. pteronyssinus*, *D. farinae*, and *Euroglyphus maynei* has also been reported; but its allergenic importance is yet to be determined.⁹ This enzyme was distinguished from trypsin and chymotrypsin on the basis of its reactivity with low MW synthetic substrates, its protease inhibition profile, and its isoelectric point. It was found in both whole mite- and fecal pellet-enriched extracts, and its apparent MW, as judged by gel filtration, was estimated to be 30,000.

In this study we report the isolation and characterization of this protease from fecal pellet-enriched extracts of *D. pteronyssinus*. Physicochemical studies confirmed that this protein was distinct from mite trypsin and chymotrypsin, as previously reported.⁹ Additionally, we demonstrate that the enzyme is a major allergen, recognized by sera of most patients allergic to mites. For this reason, we have tentatively designated this novel proteolytic allergen, Der p 9, by convention.

METHODS

Extracts and chemicals

Extracts of *D. pteronyssinus* were prepared from spent growth medium (SGM) devoid of mites, as described previously.¹⁰ General chemicals and synthetic protease

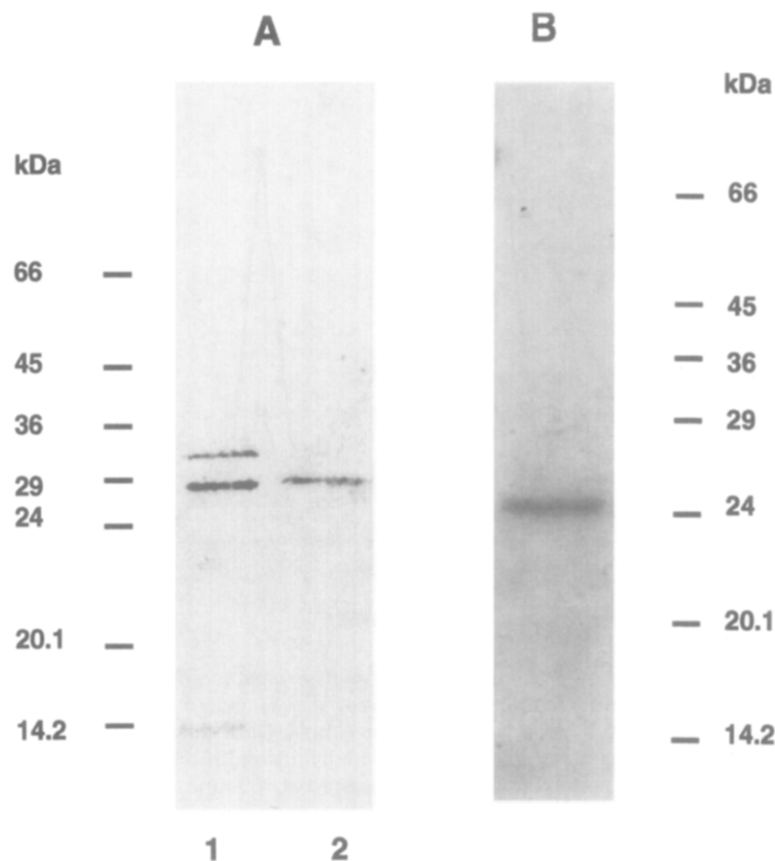


FIG. 2. SDS-PAGE analysis of isolated proteases. **A**, Lane 1 shows fraction 1 from Fig. 1 eluting between pH 4.5 and 9, and lane 2 shows fraction 2 eluting between pH 9 and 11. Lane 1 contains a mixture of the 30 kD trypsin (Der p 3) and the 28 kD protease (Der p 9) and a minor band at 14 kD. Lane 2 shows the 28 kD protease. **B**, Purified 25 kD Der p 6 chymotrypsin eluted by 0.3 mol/L phosphate buffer, pH 6.5, from a hydroxyapatite column (see Fig. 3).

substrates were purchased from Sigma Chemical Company (St Louis, Mo.), unless otherwise stated.

Determination of protease activity

Chymotrypsin and trypsin activities were determined by using succinyl-ala-ala-pro-phe p-nitroanilide (SA₂PFpNA), succinyl-ala-ala-pro-leu p-nitroanilide (SA₂PLpNA), and N-benzoyl-arginine p-nitroanilide (BAPNA), respectively, as described previously.¹¹⁻¹³ The ability of mite enzymes to hydrolyze elastase substrates was determined by using elastin-orcin and succinyl-ala-ala-ala p-nitroanilide (SA₃pNA).¹⁴ Specific activities, obtained with synthetic substrates, were expressed in units where 1 unit represented the amount of p-nitroaniline released in nanomoles per minute per milligram of protein.

Assay of collagenase activity

Digestion of type III collagen from calf skin (50 µg) by mite protease (2 µg) was performed for 30 minutes at 37° C in 200 µl of 0.05 mol/L Tris-HCl (pH 8.0). Control experiments were performed with collagenase from *Clostridium histolyticum*, and digestion in all samples was confirmed by SDS-PAGE as described previously.¹⁵ Gels

(13%) were stained with Coomassie Blue R-250, and MW was determined after reference to a standard curve constructed by using standard proteins.

Soybean trypsin inhibitor (SBTI) affinity chromatography

Sephacrose 4B (Pharmacia, Uppsala, Sweden) was activated with CNBr and then coupled with SBTI at a concentration of 5 mg/ml of packed gel. The matrix was equilibrated with 0.1 mol/L Tris buffer, pH 8, and affinity chromatography was performed at 4° C with the same buffer. Bound material was eluted with 0.1 mol/L glycine, adjusted to pH 2.8 with HCl, and immediately neutralized with solid Tris base.

Isolation of the novel protease, Der p 9

SGM was extracted in 0.01 mol/L phosphate buffer, pH 7.2, containing 0.15 mol/L NaCl, and extensively dialyzed against 0.1 mol/L Tris buffer, pH 8.0. It was then adsorbed batchwise onto diethylaminoethyl cellulose (DE53; Whatman, Maidstone, England) previously equilibrated in the same buffer for 1 hour at 4° C. The

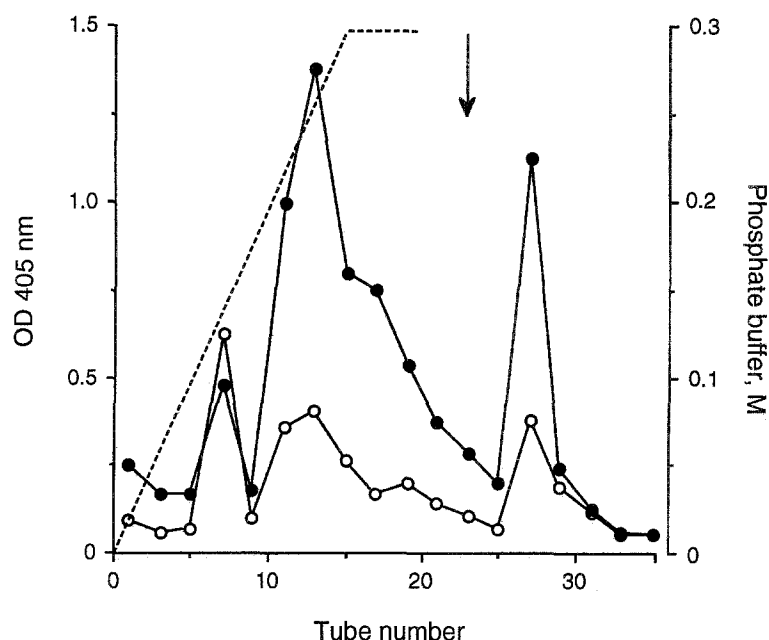


FIG. 3. Purification of the mite chymotrypsin by hydroxyapatite chromatography of copper-chelating Sepharose-bound material. Bound material was eluted with a phosphate-buffer gradient (dashed line) to 0.3 mol/L, followed by a 1 mol/L NaCl wash indicated by the arrow. Proteolytic activities of samples were determined by using SA₂PLpNA (filled circles) and SA₂PFpNA (open circles), and hydrolysis was measured at 405 nm.

unbound fraction was collected by filtration on a sintered glass funnel and applied to an SBTI-Sepharose 4B affinity matrix. The bound material was eluted with 0.1 mol/L glycine-HCl, pH 2.8, and immediately neutralized before it was assayed for enzyme activity. Fractions were pooled on the basis of specific proteolytic activity, adjusted to pH 4.5, and further purified on a 5 ml cation-exchange column (S-cartridge; Bio-Rad, Sydney, Australia) previously equilibrated with 0.1 mol/L sodium acetate buffer, pH 4.5, at room temperature. The unbound fraction was collected, and the bound fraction was eluted with distilled water adjusted to pH 11.0 with ammonia. This material was then neutralized and applied to a benzamidine Sepharose 6B affinity chromatography column (Pharmacia), as described previously.² The unbound material was then collected and assayed for protease activity.

Isolation of Der p 1, Der p 2, Der p 3, and Der p 6

Der p 1 and Der p 2 were isolated from SGM by monoclonal antibody affinity chromatography as described previously.⁴ Mite trypsin was isolated from SGM either by benzamidine affinity chromatography and chromatofocusing as described previously² or by a combination of diethylaminoethyl cellulose anion-exchange chromatography, SBTI-Sepharose 4B affinity chromatography, and cation-exchange chromatography as described previously. The mite chymotrypsin, Der p 6, was

isolated from the DE53-bound material obtained in the process of purification of Der p 9. Briefly, the DE53-bound material was eluted with 1 mol/L NaCl, adjusted to pH 6.5 with HCl, and then applied to a copper-chelating Sepharose matrix previously equilibrated with 0.1 mol/L sodium acetate buffer, pH 6.0, containing 1 mol/L NaCl at 4° C. Bound material was removed with 1 mol/L glycine adjusted to pH 6.5 with 1 mol/L sodium hydroxide, dialyzed against 0.1 mol/L phosphate buffer at the same pH, and applied to a 5 ml hydroxyapatite column (HTP cartridge, Bio-Rad) equilibrated with the same buffer. Bound material was eluted with a gradient to 0.3 mol/L phosphate buffer, pH 6.5, followed by a 1 mol/L NaCl wash. The 0.3 mol/L phosphate fraction and the 1 mol/L NaCl fraction containing chymotryptic activity were pooled, and the HTP step was repeated after appropriate dilution.

Physicochemical methods

The protein concentrations of individual samples were determined by using the Lowry modification of the Folin assay with bovine serum albumin as the standard.¹⁶ The N-terminal amino acid sequence of Der p 9 was determined after a final purification step by reverse-phase high-performance liquid chromatography on a 50 mm × 4.6 mm RP 300 column (Brownlee Labs Inc., Santa Clara, Calif.). Bound material was eluted with a linear gradient of acetonitrile (0.1% vol/vol trifluoroacetic acid in distilled water–0.09%

vol/vol trifluoroacetic acid–60% vol/vol acetonitrile) and then sequenced as described previously.¹⁷ Sequence homology searches were performed with the Swiss Protein and Protein Information Resource databases and the Basic Local Alignment Search Tool searching algorithm.¹⁸ The molecular mass of Der p 9 was determined by online electrospray ionization mass spectroscopy as described previously.^{19, 20}

Determination of allergenicity

RAST assays were performed as described previously²¹ with iodine 125-labeled anti-human IgE (Pharmacia) and CNBr-activated paper disks optimally coated with allergen. The frequencies of IgE reactivity were determined by using sera ($n = 35$) from both adults and children, which had been previously selected on the basis of their reactivity to whole mite in RAST, and data were expressed as percent radiation bound. Sera from adult and child donors who were not allergic to mites ($n = 12$) and from a donor with hyper-IgE syndrome (100,000 U/ml) were also assessed to control for nonspecific binding. A positive response for each allergen was defined as a percent radiation bound greater than the sum of the mean radiation bound obtained with the control sera and twice the standard deviation. RAST inhibition was performed by using CNBr-activated disks coupled with Der p 9. Pooled mite allergic serum was diluted to give 50% of the maximum bindable ¹²⁵I-labeled IgE and incubated with varying concentrations of inhibitor for 2 hours at room temperature before addition of the allergen-coupled disks. Statistical correlations were determined by using the Kaleidagraph computer program (Synergy Software, Reading, Pa.).

RESULTS

Isolation of Der p 9 from *D. pteronyssinus* SGM

SGM was absorbed batchwise onto DE53 at pH 8.0, and the unbound fraction was then absorbed onto an SBTI–Sephrose 4B affinity matrix. The bound material, which eluted at acid pH, was shown to cleave SA₂PLpNA, SA₂PFpNA, and BApNA. Fractions containing the proteases were pooled and concentrated by cation-exchange chromatography at pH 4.5. The bound material was eluted with distilled water at pH 11.0, and two fractions were obtained (Fig. 1). The first, which eluted between pH 4.5 and 9.0, was found to contain two protein bands with apparent MW of 28,000 and 30,000 as judged by SDS-PAGE (Fig. 2, A). The proteins in this fraction cleaved SA₂PLpNA, SA₂PFpNA, and BApNA (Fig. 1). The protein with an apparent MW of 30,000 d present in the fraction eluted between pH 4.5 and 9.0 was judged to be trypsin on the basis of its cleavage of BApNA and subsequent purification on benzami-

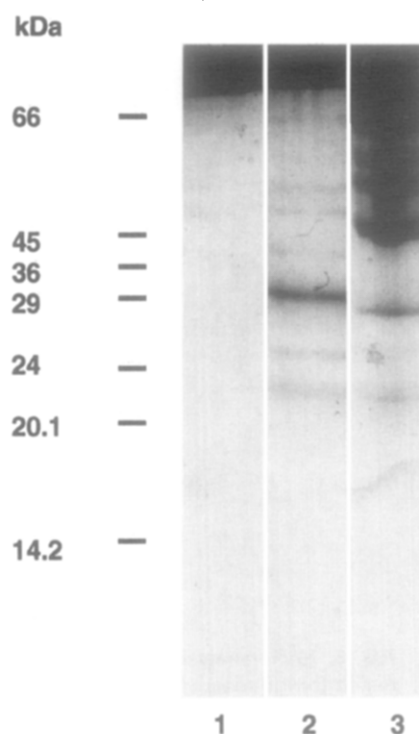


FIG. 4. Degradation of type III collagen (lane 1) from calf skin by clostridial collagenase (lane 2) and Der p 9 (lane 3) as judged by SDS-PAGE.

dine affinity chromatography. The material eluting between pH 9.0 and 11.0 contained a protein with an apparent MW of 28,000 d (Fig. 2, A) and cleaved SA₂PLpNA and SA₂PFpNA only (Fig. 1). This material was used in all subsequent studies.

The purified Der p 9 protease appeared to be stable, but degradation was found to occur in the presence of mite trypsin at 4° C (data not shown). The purified protease was found to have a specific activity of 377 units, which represented an increase of 899-fold over that found in the original SGM starting material. One hundred forty milligrams of purified Der p 9 was isolated from 5 gm of SGM.

Der p 6 was isolated as a by-product of the Der p 9 isolation scheme. It was eluted from the DE53 with 1 mol/L NaCl and absorbed onto a copper-chelating Sepharose affinity column. The bound material was eluted with 1 mol/L glycine and applied to a hydroxyapatite matrix. Bound material was eluted with a phosphate-buffer gradient, and two proteolytically active peaks were detected, which eluted with 0.3 mol/L phosphate and 1 mol/L NaCl, respectively (data not shown). The two peaks were pooled and reappplied to the hydroxyapatite matrix with the same phosphate gradient. The resulting 0.3 mol/L phosphate fraction and the 1 mol/L NaCl fraction possessed chymo-

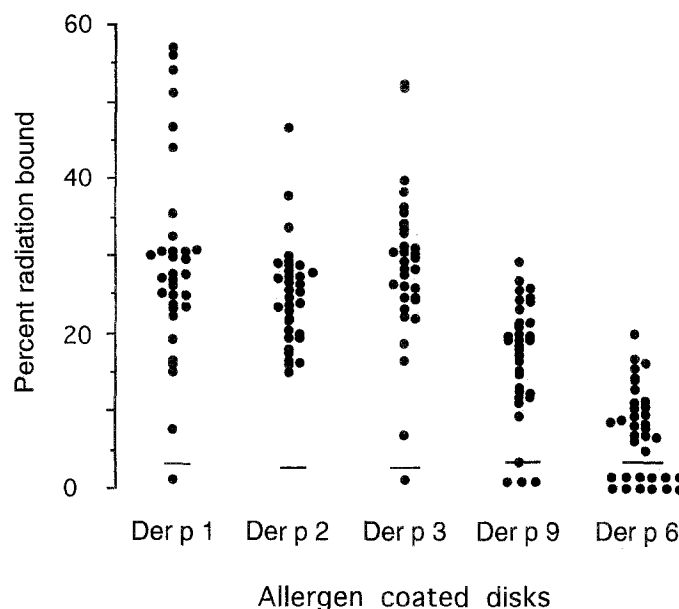


FIG. 5. RAST analysis of individual mite allergens. Sera from 35 patients allergic to mites were tested for IgE reactivity to Der p 1, Der p 2, Der p 6, and Der p 9. Data for Der p 3 were obtained from the same panel but reported previously.² Conditions for the assay were similar for both experiments. Individual patients' scores are shown, and lines represent mean radiation bound added to twice the standard deviation for each disk type obtained by using sera from a non-mite-allergic panel ($n = 12$).

trypsin-like activity as determined by cleavage of the substrate SA₂PFpNA (Fig. 3) and contained a protein with an apparent MW of 25,000 d (Fig. 2, B).

Enzymatic properties of Der p 9

The purified Der p 9 protease did not cleave either elastin-orcein or the elastase-specific substrate SA3pNA (data not shown). However, Der p 9 was shown to degrade collagen, and at least seven fragments were detected as judged by SDS-PAGE (Fig. 4). The MW spectrum obtained with the allergen differed from that obtained with Clostridial collagenase.

Physicochemical properties of Der p 9

The N-terminal sequence of the 28 kd protease was determined after further purification on a reverse-phase column by gas phase Edman degradation, and the data obtained are shown in Table I. Eighteen residues were determined, of which 11 were identical with corresponding residues in Der p 3, and 10 in Der p 6. Sequence homology searches indicated that the protease was 72% similar (identical residues combined with chemically similar residues) to the group 3 and 6 allergenic proteases^{2,3} and 83% similar to invertebrate serine proteases from the Kamchatka crab (*Paralithodes camtschatica*) and the crab beetle (*Chio-*

noectes opilio).^{22,23} The molecular mass was found to be 23,780 d as judged by electrospray mass spectroscopy.

Allergenicity of Der p 9

To determine whether the 28 kd protease was allergenic, RAST studies were performed with sera from 35 donors allergic to mites and 12 nonallergic donors who had previously been tested for reactivity with whole mite extract and Der p 3.² Each serum sample was analyzed for IgE binding to Der p 1, Der p 2, Der p 6, and the novel serine protease (Fig. 5). These studies showed that the novel protease was allergenic. The percent frequencies of reactivity obtained for Der p 1, Der p 2, Der p 3, Der p 6, and Der p 9 were 96, 100, 97, 65, and 92, respectively. The mean values of percent radiation bound for these allergens were 29, 24, 28, 8, and 17, respectively. Linear regression analysis was performed to determine whether there was any relationship between the responses to the different allergens. These studies revealed a significant correlation between the 28 kd allergen and Der p 3 ($r = 0.63$, $p < 0.0001$) and Der p 6 ($r = 0.57$, $p < 0.001$). In contrast, responses obtained with the 28 kd allergen, Der p 3, and Der p 6 did not correlate with either Der p 1 or Der p 2, although responses to Der p 1 correlated with those of Der p 2 ($r =$

TABLE I. N-terminal sequences of mite serine proteases and homologous proteases

Protease	Sequence																	
	1																18	
Der p 9	I	V	G	G	S	N	A	S	P	G	D	A	V	Y	Q	I	A	L
Der f 3	I	V	G	G	V	K	A	Q	<u>A</u>	G	D	X	P	Y	Q	I	<u>S</u>	L
Der p 3	I	V	G	G	E	K	A	L	<u>A</u>	G	<u>E</u>	X	P	Y	Q	I	<u>S</u>	L
Der f 6	(A)	(V)	G	G	Q	D	A	D	L	<u>A</u>	<u>E</u>	A	P	F	Q	I	<u>S</u>	L
Der p 6	(A)	<u>I</u>	G	X	Q	(P)	A	<u>A</u>	E	(A)	(E)	A	P	F	Q	I	(S)	L
Kamchatka protease	I	V	G	G	<u>T</u>	E	V	<u>T</u>	P	G	<u>E</u>	I	P	Y	Q	<u>L</u>	<u>S</u>	L
Crab beetle protease	I	V	G	G	Q	E	<u>A</u>	<u>T</u>	P	H	T	W	<u>V</u>	H	Q	<u>Y</u>	<u>A</u>	L

One letter code used throughout. X indicates that the residue could not be determined. Letters in parentheses indicate uncertainty. Residues showing identity with the 28 kd protease are boxed, whereas those residues considered to be chemically similar by the searching algorithm are underlined. Sequences were obtained from previous studies.^{2-4, 22, 23}

0.62, $p < 0.001$). The allergenic cross-reactivity between Der p 9 and the other mite serine proteases was investigated by RAST-inhibition. Little allergenic cross-reactivity was observed with Der p 6, but 20% inhibition was observed with Der p 3 at the highest concentration studied (Fig. 6).

DISCUSSION

A novel, polymorphic serine protease with allergenic activity was isolated from SGM of *D. pteronyssinus*. It was clearly differentiated from both the Der p 3 tryptic allergen² and the Der p 6 chymotryptic allergen³ on the basis of charge, N-terminal amino acid sequence, and substrate specificity. The protease was shown to be allergenic and was therefore designated Der p 9. This protease represents the fourth mite protease to be described, and these results and previous data^{1-3, 8-10} highlight the importance of feces-derived hydrolytic enzymes in mite-induced allergy.

Der p 9 was shown to migrate as a single band on SDS-PAGE with an apparent MW of 28,000 d. However, electrospray mass spectroscopy indicated the molecular mass was 23,780 d, a value similar to that deduced from the complementary DNA studies of Der p 3, namely, 24,985 d.⁷ An examination of the first 18 residues of the N-terminal sequence indicated that it was similar to both the group 3 and group 6 mite allergens, invertebrate chymotrypsins, and collagenolytic serine proteases.²³ However, unlike the group 3 and group 6 mite allergens and the majority of serine proteases from other species, Der p 9 con-

tained a nonpolar hydrophobic valine residue at position 13 in the polypeptide chain, rather than the typical proline residue. Interestingly, a valine residue is also found in the corresponding position of the collagenolytic serine protease from the crab beetle (*Chionoecetes opilio*),²³ but the significance of this substitution remains unclear.

Der p 9 was shown to be similar to chymotrypsins and cathepsin G-like enzymes from both vertebrate and invertebrate sources as judged by cleavage of substrates containing either C-terminal phenylalanine or leucine residues.^{12, 13, 23-25} Like collagenolytic serine proteases from other invertebrate species,^{22, 23} the Der p 9 protease also degraded native collagen, although the spectrum of cleavage products differed from that seen with bacterial collagenase, suggesting a different substrate specificity to the metalloproteinase enzymes. In this regard, the specificity of Der p 9 may be analogous to that of the enzyme from the insect *Hypoderma lineatum*, which cleaves at the C-terminal side of leucine rather than the X-glycine bond in the collagen motif, proline-X-glycine-proline (where X represents any amino acid residue).²⁶ Der p 9 has probably evolved to participate in the digestion of collagenous substrates in the form of human skin scales.

RAST studies showed that 92% of patients allergic to mites had specific IgE antibodies to Der p 9, a value comparable to that observed with Der p 1, Der p 2, and Der p 3. In contrast, the frequency of reactivity to Der p 6 was lower than that seen with the previously mentioned allergens, suggesting that it is less potent than either of the

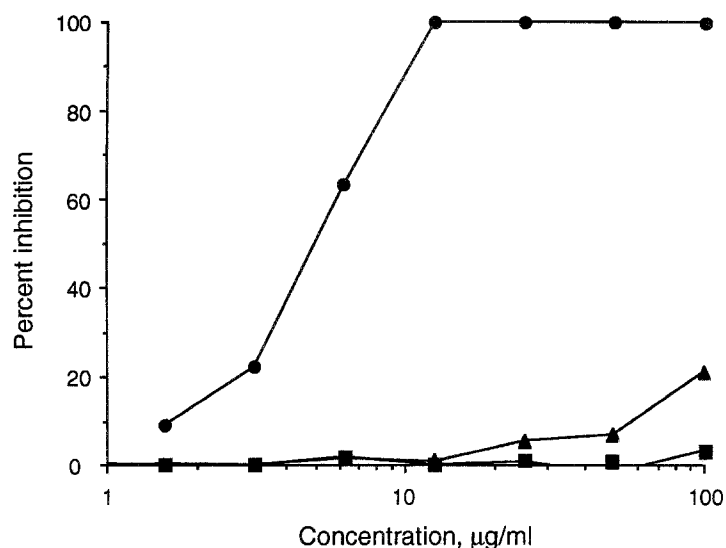


FIG. 6. RAST-inhibition analysis showing allergenic cross-reactivity between Der p 9 and either Der p 6 or Der p 3 or Der p 9 itself as a control. Disks were coated with Der p 9, and RAST inhibition was performed with Der p 9 (filled circles), Der p 3 (filled triangles), and Der p 6 (filled squares).

other serine protease allergens. Interestingly, however, the frequency of reactivity seen with Der p 6 in our study (65%) was slightly higher than the 41% reactivity reported by Yasueda et al.³ Further examination of the RAST data revealed significant correlations between responses obtained with the 28 kD allergen and both Der p 3 and Der p 6, suggesting either similar degrees of exposure to the allergens or immunologic cross-reactivity. However, little, if any, correlation was found between the reactivity to the mite serine proteases and either Der p 1 or Der p 2, but the reasons for these observations are unclear.

The RAST-inhibition data reported here suggest limited immunologic cross-reactivity between Der p 9 and Der p 3 but not between the novel allergen and Der p 6. In this regard, we have previously shown that material isolated from *D. farinae* by affinity chromatography with a monoclonal antibody raised against Der f 3 cleaved both trypsin and chymotrypsin/cathepsin G-like substrates,⁹ suggesting the isolation of at least two biochemically distinct but immunologically related enzymes. In addition, other workers,⁷ using this same antibody, have reported the isolation of a product appearing as a 28/30 kD doublet by SDS-PAGE. Because trypsin has a MW of 30,000 d in this system, it is feasible that the component with an MW of 28,000 d corresponds to the Der p 9 allergen reported here. That only one sequence was obtained in the latter study⁷ may reflect our

anecdotal findings that Der p 9 protease is susceptible to degradation by Der p 3.

Given the biochemical relatedness of the three serine protease allergens, the discrepancy between the high reactivity to Der p 3 and Der p 9 and the relatively low reactivity to Der p 6 in RAST studies is interesting. Whether these differences reflect the relative concentrations of the mite serine proteases in dust or the immunochemical relatedness of the group 3 and group 9 allergens is at present unclear. Measurement of the individual proteases in dust, together with cloning studies, to facilitate a comparison of the three serine protease sequences and epitope mapping studies, may help resolve the issues raised by these observations.

In conclusion, we have shown that feces-enriched extracts of *D. pteronyssinus* contain a chymotrypsin- or cathepsin G-like serine protease with the ability to degrade collagen. It had a molecular mass of 23,780 d and was shown to be allergenic. It demonstrated limited cross-reactivity with the mite trypsin allergen Der p 3 but not with Der p 6. This proteolytic allergen can now be added to the existing list of dust mite allergens that, by virtue of their biochemical activity, may enhance interaction with the immune system.²⁷

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