

Purification and IgE binding capacity of Der s 3, a major allergen from *Dermatophagoides siboney*

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Summary

Background Sensitization to the house dust mite *Dermatophagoides siboney* has been demonstrated in asthmatic patients. Previously, *Dermatophagoides siboney* group 1 and group 2 allergens, named Der s 1 and Der s 2, respectively, have been purified.

Objectives The aim of this study was to purify and to study the IgE reactivity of a 30 kDa component, suspected to correspond to group 3 allergens.

Methods The protein was purified by affinity chromatography using anti-Der f 3 monoclonal antibodies and semi-preparative SDS-PAGE. The IgE binding capacity of the purified fractions was tested with sera from 106 mite-sensitive asthmatic patients using a modified chemiluminiscent method.

Results Affinity chromatography resulted in fractions containing the 30 kDa component which was further purified to homogeneity by SDS-PAGE. Seventy-three per cent of the sera showed IgE reactivity to this protein, indicating that it is a major allergen. The protein also reacted with anti-Der f 3 polyclonal antibodies and had tryptic activity. There were differences in the reactivity to Der s 3 according to the age of the patients.

Conclusion Based on the frequency of IgE reactions and the reactivity with antibodies directed to Der f 3, it is proposed to name this 30 kDa allergen from *D. siboney*, Der s 3.

Keywords: mite allergens, affinity-chromatography, chemiluminescence, Der s 3, *Dermatophagoides siboney*, SDS-PAGE

Clinical and Experimental Allergy, Vol. 27, pp. 700–704. Submitted 18 May 1996; revised 12 October 1996; accepted 19 December 1996.

Introduction

Dermatophagoides siboney has been commonly found in house dust of asthmatics in the Caribbean area [1,2]. Sensitization to this mite species has been demonstrated through skin-prick tests and *in vitro* IgE tests [3]. So far, at least 13 components with IgE binding capacity from *D. siboney* have been identified and two of them, i.e. Der s 1 and Der s 2, have been purified by affinity chromatography using crossreacting monoclonal antibodies to group 1 and group 2 *Dermatophagoides* allergens, respectively, [4].

Allergens from group 3 have been isolated and characterized from *D. farinae* and *D. pteronyssinus*. They show polymorphism with molecular weights ranging from 25 to 34 kDa [5,7,8,20]. In a previous study of *D. siboney*, a

protein of 30 kDa was found which reacted with more than 30% of 35 sera from atopic mite sensitive patients. This protein was proposed to correspond to group 3 allergens [4].

The aim of this study was to purify the 30 kDa allergen from *D. siboney* and to study its IgE binding capacity against a panel of sera from mite sensitive asthmatics.

Materials and methods

Purification of Der s 3

Freeze-dried extract from whole mite culture [4] was reconstituted in phosphate buffered saline (PBS) pH 7.4 and adsorbed to a Sepharose CNBr 4B affinity column (Pharmacia, Sweden) coupled with 6D7 monoclonal antibody reacting to Der f 3 (kindly supplied by Martin D. Chapman, Charlottesville, USA) according to the procedure prescribed by the manufacturer. After adsorption, proteins

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were eluted by 50% ethylene glycol in 0.005 M glycine pH 10.0. The fractions corresponding to the protein peak at 280 nm were pooled, desalted by gel filtration on a G-25 column (Pharmacia, Sweden) and vacuum dried (Speed Vac, USA) overnight. The purity of the pooled fraction was analysed by SDS-PAGE under non-reducing conditions and the bands detected by silver staining.

Further purification of the 30 kDa band was achieved by semi-preparative SDS-PAGE on 1 mm 12.5% gel. After electrophoresis, a strip of 2 mm width along the entire gel shown to correspond to 30 kDa by the use of coloured molecular weight markers (BioRad, USA), was cut out. This polyacrylamide strip was cut into 1 mm² pieces and extracted with 0.005 M ammonium bicarbonate by an homogenizer. After centrifugation to remove non-soluble material, the supernatant was vacuum dried. The purity of the preparation was checked by SDS-PAGE developed by silver-staining and by western blotting [4] using a pool of sera from sensitized patients [3], 6D7 MoAb and rabbit anti Der f 3 (kindly supplied by Dr W.R. Thomas, Perth, Australia).

Patients and sera

Sera from 106 asthmatic patients, who showed positive skin-prick tests and/or *in vitro* IgE tests to a panel of mite allergens, were collected and processed as previously described [3].

IgE binding capacity of the purified fraction

To check the IgE-binding frequency of the purified protein, a modification of an IgE chemiluminescent assay (MagicLite, ALK, Denmark) was used. Dried protein was reconstituted in PBS pH 7.4 at 0.3 mg protein/mL (BioRad Protein Assay, using bovine serum albumin as standard) and applied to discs of 5 mm diameter (10 µL/disc) cut from 0.2 µm nitro-cellulose membranes (Pharmacia, Sweden). After drying at room temperature, the discs were blocked in PBS containing 0.5% Tween-20 at 37°C during 1 h and incubated overnight in duplicate with each of 106 individual patient sera diluted 1:5 in PBS-Tween. Different types of controls were used: purified Der s 3 and non-mite sensitized human sera (background controls), birch pollen extract and Swedish birch sensitive human sera (positive control), and birch pollen extract and Cuban mite sensitive human sera (negative control). Next day the discs were washed with PBS-Tween and incubated 30 min at room temperature with 100 µL of anti-IgE chemiluminescent reagent (ALK, Denmark) per disc. The reactions were measured by MagicLite Analyzer II (Ciba Corning, Germany) and the results expressed as a ratio of the sample value and background control. Ratios higher than 2 were considered positive. The

results of IgE binding were analysed by non-parametric tests using StatView software (Abacus, USA).

Enzymatic activity

A sample of the purified preparation was reconstituted in distilled water (0.3 mg protein/mL) and analysed against N-benzoyl-DL-arginine-2-naphtylamide and N-glutaryl-phenylalanine-2-naphtylamide substrates for trypsin- and chymotrypsin-like activity, respectively (ApiZym, Bio Mérieux, France).

Results

Purification of Der s 3

Affinity chromatography using 6D7 anti Der f 3 monoclonal antibodies showed a well defined peak eluted by ethylene glycol. By Coomassie blue staining, the analysis by SDS-PAGE of pooled fractions of different regions of the peak, showed a relative pure protein band of 30 kDa, especially in the fractions eluted at the tail. Silver staining, however,

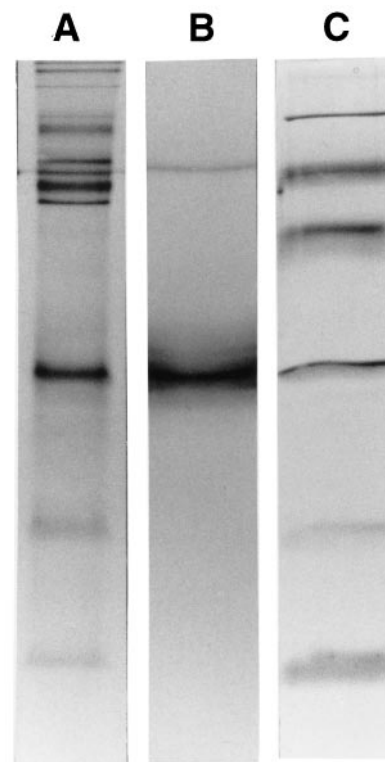


Fig. 1. SDS-PAGE of Der s 3 allergen. Lane A: affinity chromatography fraction by monoclonal anti-Der f 3 antibody; Lane B: purified preparation after semi-preparative electrophoresis; Lane C: molecular weight markers (94, 67, 43, 30, 20 and 14.4 kDa).

demonstrated different contaminants in the affinity purified materials. Further purification was achieved by isolating and extracting the 30 kDa band from semi-preparative SDS-PAGE gels. When re-analysed by electrophoresis and silver-stained, this preparation showed a high degree of purity (Fig. 1).

Western blotting of this preparations showed reactivity with a serum pool of mite-sensitive patients, as well as reactivity with anti Der f 3 poly- and monoclonal antibodies (data not shown).

IgE binding

As determined by chemiluminescent reactions on nitrocellulose discs, Der s 3 reacted positively with 73% of the 106 patient sera analysed (Fig. 2). Among the positives, 82% showed a binding ratio higher than 4, indicating a high degree of reactivity with Der s 3. The backgrounds found in all the negative controls were very low, indicating no non-specific binding of IgE to the solid phase. Birch pollen allergen showed high IgE reactivity in a selected group of birch-sensitive patients used as positive controls.

All the sera which reacted to Der s 3 in the previous Western blotting experiments [4], also reacted with the purified preparation used in this study. However, some of the previous negatives were actually found to give a positive reaction to the purified Der s 3.

There was a significant difference in the reactivity to Der s 3 depending of the age of the patients. Two of 10 sera (20%) from children less than 15 years old, 9/18 sera (50%) from individuals between 15 and 20 years old and 66/78 sera (85%) from patients above 21 years of age, reacted with

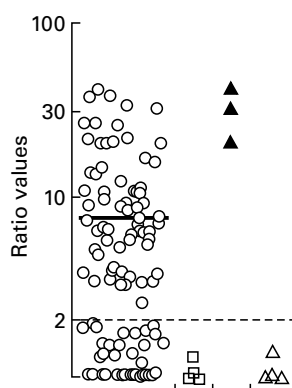


Fig. 2. IgE binding to Der s 3 in mite sensitized individuals ○, expressed as the ratio sample/background, in the population studied. The horizontal solid line represents the median value for Der s 3; the dashed line represents the cut-off of the negatives. IgE binding to Der s 3 in non-sensitized individuals □, to birch by birch-sensitized individuals ▲, and to birch allergen by individuals not sensitized to birch △, are shown.

Der s 3. However, no correlation was observed between the intensity of IgE binding and the age of patients.

Enzymatic activity

The purified Der s 3 showed tryptic-like but no chymotryptic-like activity.

Discussion

In a previous study of *D. siboney* we found a distinct band of 30 kDa. Thirty per cent of 35 patients showed IgE binding to this band. We proposed that this protein was the group 3 allergen of this species [4].

In this paper we describe the purification of an allergen from *D. siboney*. Due to the molar weight similar to that of Der f 3 and Der p 3, its binding to Der f 3 specific monoclonal and polyclonal antibodies, and to the degree of IgE binding in a large panel of sera, it should be considered a major allergen from this mite. We propose to name it Der s 3.

Allergens of group 3 from *Dermatophagoides* have been isolated and studied in *D. farinae* [5,8] and in *D. pteronyssinus* [6,7]. These allergens correspond to proteins of 21, 28–30 and 32 kDa, which like Der s 3 possess tryptic activity [8–10]. Similar results have been reported for *Euroglyphus maynei* [11].

According to the high degree of crossreactivity found between *D. siboney* and *D. farinae* [12] it was not surprising that the use of monoclonal antibodies directed against Der f 3 reacted with a corresponding protein in *D. siboney* and could be used in the first step of purification. Ongoing studies on the crossreactivity of individual allergens between *D. siboney* and other mite species by western blotting inhibition have shown that this 30 kDa band is inhibited by low concentrations of *D. farinae* than *D. pteronyssinus* or *D. microceras* extracts (data not shown). Moreover, the allergenic similarity between Der f 3 and the 30 kDa protein from *D. siboney* was supported by the recognition of this protein by polyclonal anti Der f 3.

Thomas *et al.* [9] could not find any reactivity to a *D. pteronyssinus* extract in western blotting using a monoclonal antibody directed to Der f 3 but found a strong reactivity when polyclonal antibodies against Der f 3 were used. This indicates that at least one epitope in Der f 3 is specific and not shared with Der p 3. Similar monoclonal antibodies specific for Der p 1 and 2 and Der f 1 and 2 form the basis for assays for determination of these allergens. The degree of homology between the N-terminal sequences of Der p 3 and Der f 3 (78%) [7] is less than observed between Der p 2 and Der f 2 (88%) [13] or between Der p 1 and Der f 1 (81%) [14]. Moreover, a higher correlation in the concentration of IgE antibodies to Der p 1 and Der f 1 ($r = 0.95$)

and Der p 2 and Der f 2 ($r = 0.98$) than between Der p 3 and Der f 3 ($r = 0.4$) has been found [15], suggesting a lower degree of crossreactivity between the group 3 molecules than between group 1 and 2 allergens. Complete sequence analysis of Der 3 allergens, including Der s 3, will contribute to a better understanding of the crossreactivities between group 3 mite allergens.

Beside Der p 3 and Der f 3 which possess tryptic activity, at least two more proteins have been found in the 30 kDa region in extracts of *Dermatophagoides* species, which showed chymotrypsin and elastase activity, respectively, [16]. However, monoclonal antibodies to Der f 3 did not react to the protein with elastase-like activity and only to a limited extent to the chymotrypsin-like enzyme [17]. The fact that our preparation showed only trypsin-like activity and no chymotrypsin-like activity confirms that it is a Group 3 allergen.

In a previous study of a small sample of patients ($n = 35$) we found that about 30% of them showed IgE reactivity against the 30 kDa protein when *D. siboney* extract was fractionated by SDS-PAGE and immunoblotted [4]. This figure increased to 73% using the purified preparation as used in this paper, probably due to the lower concentration of Der s 3 when whole crude extract was fractionated by SDS-PAGE. There are some controversial findings on the frequency of IgE reactivity to allergens of group 3. Heymann *et al.* found reactions in only 16% of their patients [5], Yasueda *et al.* in 60% [15], Tovey and Baldo [18] and Ando *et al.* [8] in 70% and Thomas in 38% of children and 83% of adults [9]. The reactivity to a 30 kDa protein from *D. pteronyssinus* was found also to increase with the age of children and adolescents [19]. Variation between populations used in different studies could be one of the explanation for the variability in the percentage of reactivity observed to group 3 allergens. According to our results, only 20% patients less than 15 years old and 39% of patients younger than 20 years old, reacted to Der s 3. It could be that Der s 3 has lower immunogenicity or its concentration in the house dust or in the air is lower than that of other major allergens. Then, longer exposure periods might be necessary for sensitization. The different methods used for IgE determination, the variation in concentration (up to 149-fold) of Der 3 in different extracts [17], the degree of IgE affinity and the amount of specific IgE present in the population studied [9,19] might also explain the differences noted.

Acknowledgements

This study was supported by the Department of Paediatrics, Linköping University Hospital and Swedish Institute, Sweden. The authors wish to thank Professor Bengt Björkstén for his continuous support and helpful discussions,

and to Kerstin Hagersten and Lisbeth Hjälle for their help in the laboratory.

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