



Glutathione *S*-transferase a major allergen of the house dust mite, *Dermatophagoides pteronyssinus*

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Abstract

Recently, we cloned a new *Dermatophagoides pteronyssinus* allergen homologous with glutathione *S*-transferase. IgE radioimmunoassays against the *Escherichia coli* lysate of the recombinant clone reveal that 40% of mite allergic subjects recognize the mite glutathione *S*-transferase allergen. Of these sera, greater than 50% have reactivity with the recombinant mite glutathione *S*-transferase that is greater than 10 times the result observed with a normal control. Immunoblotting studies with sera from patients that recognize the recombinant protein reveal IgE binding to a 26-kDa protein on immunoblots of reduced mite protein extracts. The 26-kDa IgE-binding band observed on immunoblots of reduced mite proteins, corresponding to the cloned protein, is a separate and unique allergen from the 25-kDa *Der p* I as the apparent electrophoretic molecular mass of *Der p* I shifts from 25 to 30 kDa under conditions of reduction.

Keywords: Allergen; Glutathione *S*-transferase; Mite; IgE

1. Introduction

The house dust mite, *Dermatophagoides pteronyssinus*, has long been recognized as a major cause of allergy and asthma among sensitized individuals [1–3]. There are at least 32 IgE-binding house dust mite proteins recognized by the sera of allergic subjects [4]. Of these, approximately six have a high frequency of recognition by allergic subjects and are considered to be major allergens. Preparation and analysis of cDNA libraries of *D. pteronyssinus* has led to the characterization of a number of mite allergens: *Der p* I [5], a 14-kDa polypeptide [6], *Der p* II [7], a 22-kDa polypeptide [8] and *Der p* III [9]. Recently, we have cloned a 25.589-kDa allergen with sequence homology to glutathione *S*-transferases [10]. SDS-PAGE and protein blotting studies have revealed a number of allergens with molecular masses between 25 and 30 kDa, in particular *Der p* I, and it is therefore im-

portant to be able to distinguish the 25.589-kDa mite glutathione *S*-transferase allergen, from the 25.371-kDa *Der p* I allergen. IgE-binding studies demonstrate that the mite glutathione *S*-transferase is a major allergen and the results of these studies are presented in this paper.

2. Materials and methods

2.1. Sera, mite extracts and preparation of *E. coli* lysates of λ gt11 clones

Sera from mite skin-prick test positive patients were obtained from Dr Richard Baker (Macquarie St, Sydney, Australia) and the Allergy Clinic, Royal North Shore Hospital (Sydney, Australia). Cord serum was obtained from the Obstetrics Department of Royal North Shore Hospital. Preparation of mite extracts was as previously described [11]. *Escherichia coli* lysates of an induced lysogen of the λ gt11 cDNA clone encoding the mite glutathione *S*-transferase allergen were prepared as previously described [10].

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2.2. IgE radioimmunoassays

Escherichia coli lysates of an induced lysogen of the λ gt11 cDNA clone encoding the mite glutathione *S*-transferase allergen were prepared. Nitrocellulose (0.45 μ m, Bio-rad, Richmond, CA, USA) discs were incubated with either 20 μ g of total house dust mite protein per disc or 80 μ g of total induced *E. coli* lysate per disc as determined using the Pierce BCA protein determination kit (Pierce, Rockford, IL, USA). Discs were then blocked for 1 h with 0.1% Tween 20 in phosphate-buffered saline. The discs were then assayed for IgE binding in a radioimmunoassay, using mite-allergic sera. Bound IgE antibodies were detected by probing with ^{125} I-anti-human anti-IgE (Bioclone, Sydney, Australia), 30 000 counts/min per disc. Results were expressed as the percentage uptake of the total labelled anti-human anti-IgE added. Cord serum was used as a non-IgE-containing serum control and non-mite-reactive, allergic serum was used as a normal control. Discs of non-recombinant λ gt11-induced lysates were prepared and used to determine the level of background IgE binding to *E. coli* proteins. A positive result was taken as a value greater than 3 times the value achieved with cord serum and/or greater than three times the value achieved with the control non-recombinant λ gt11 lysate.

2.3. SDS-PAGE and Western blotting

Inclusion bodies were prepared from λ gt11 clone lysates as described [12]. Inclusion bodies were dissolved in an equal volume of double strength sample buffer (120 mM Tris, 8.0% SDS (w/v), 20.0% glycerol (v/v), 2.0% bromophenol blue (w/v), pH 6.8), boiled for 10 min and the extract was electrophoresed through 6.0% SDS-polyacrylamide gels. The proteins were then transferred to nitrocellulose (0.1 μ m, Schleicher and Schuell, Germany), the nitrocellulose filters blocked and then probed with mite allergic sera followed by ^{125} I-labelled anti-human anti-IgE (Bioclone) as previously described [10]. Strips were then exposed overnight to a phosphor storage screen and the image prepared using the Imagequant programme of a Phosphorimager (Molecular Dynamics, Sunny Vale, CA, USA).

Extracts of mite were treated prior to electrophoresis as follows: treatment 1, heated (100°C for 10 min); treatment 2, not heated and reduced (room temperature for 10 min, 5.0% mercaptoethanol); and treatment 3, heated and reduced (100°C for 10 min, 5.0% mercaptoethanol); and all samples were diluted in equal volumes of sample buffer. The treated samples were electrophoresed in 9–27% gradient SDS-polyacrylamide gels and the proteins were transferred to nitrocellulose (0.1 μ m, Schleicher and Schuell) and probed with sera allergic to the mite GST allergen as described above.

3. Results

3.1. IgE-binding properties of the recombinant glutathione *S*-transferase allergen

Western blots of *E. coli* lysates of the λ gt11 clone encoding the mite GST were probed with a serum having IgE antibodies against the mite GST. The IgE antibodies bound to a protein of approximately 140 kDa corresponding to the mite GST protein fused to β -galactosidase encoded by the λ vector (Fig. 1). A non-recombinant λ gt11 control lysate was probed with the same serum sample and no IgE-antibody binding was observed (results not shown).

Sera (193) shown to give a positive result in IgE radioimmunoassays against *D. pteronyssinus* were tested against nitrocellulose discs impregnated with *E. coli* lysates of the recombinant clone and a non-recombinant λ gt11 control. Seventy-seven of the 193 sera tested (40%) gave a positive result with the *E. coli* lysate of the recombinant clone. Percent uptake values, for the 193 sera tested, ranged from 1.6 to 47.8%. Table 1 details the IgE radioimmunoassay results achieved for the eight sera shown in Fig. 2d–k. The highest percent uptake for this set of sera against the recombinant clone *E. coli* lysate was 38.2% and the lowest was 4.5%.

Of the 77 positive sera, 14% had a result between 2.5 and 5.0 times the normal control, 25% had a result between 5.1 and 10.0 times the normal control, and 53% had a result greater than 10.0 times the normal control. The remaining 8.0% of sera had percent uptakes greater

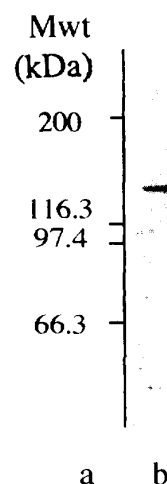


Fig. 1. Immunoblot of inclusion bodies prepared from *E. coli* lysates of the λ gt11 clone encoding the mite GST, prepared as described in Materials and methods. (a) Molecular weight markers; (b) immunoblot of λ lysate probed with a serum reactive to the mite GST allergen.

Table 1
Uptake of IgE antibodies from mite-allergic sera against the *E. coli* lysate of the recombinant *D. Pteronyssinus* glutathione *S*-transferase clone

Solid phase	% uptake ^a									
	D	E	F	G	H	I	J	K	cord	normal ^b
Recombinant mite glutathione <i>S</i> -transferase lysate	15.8 (0.6)	24.5 (0.1)	18.8 (0.7)	38.2 (0.1)	22.5 (0.5)	13.8 (0.7)	4.5 (0.2)	35.5 (0.9)	0.4 (0.1)	1.4 (0.1)
<i>D. pteronyssinus</i> extract	25.8 (3.5)	30.0 (0.9)	32.1 (2.1)	28.6 (0.8)	33.2 (0.4)	17.1 (2.1)	28.2 (3.7)	27.7 (0.3)	0.4 (0.0)	0.8 (0.1)
Non-recombinant λ gt11 lysate	1.6 (0.1)	1.1 (0.1)	1.4 (0.2)	1.3 (0.5)	1.3 (0.7)	0.5 (0.0)	0.4 (0.0)	1.6 (0.0)	0.3 (0.1)	1.2 (0.0)

Results are expressed as a percentage of the total ¹²⁵I-anti-human anti-IgE added (standard deviations are shown in brackets). All sera were tested in duplicate.

^aA value greater than 3 times the cord value and/or 3 times the value achieved with the control non-recombinant λ gt11 lysate was considered to be a positive result.

^bNon-mite-reactive, allergic serum.

than 3 times the value achieved with the cord serum, but a value less than 2.5 times the value of the normal control serum. These sera are still considered positive, although weak, as the uptake with the recombinant clone *E. coli* lysate is greater than 3 times the uptake with the non-recombinant λ gt11 control *E. coli* lysate.

3.2. Identification of the mite glutathione *S*-transferase allergen on Western blots of mite extract

Western blots of mite extracts (treatment 1) were probed with sera that gave a positive uptake in radioimmunoassays against the recombinant lysate. IgE binding to a common band of 25.589 kDa could not be discerned under these conditions (Fig. 2c). However, probing Western blots of heated, reduced mite proteins (treatment 3) with sera reactive to the cloned polypeptide revealed IgE binding to a common band with a molecular weight of approximately 26 kDa (Fig. 2d–k). The weak IgE-binding profile of serum k corresponds to the low percent uptake (4.5%) of this serum against lysates of the recombinant clone (Table 1).

3.3. Distinguishing the 25.589-kDa mite glutathione *S*-transferase allergen from the 25.371-kDa *Der p* I allergen

Electrophoretic analysis of heat-treated mite proteins suggest *Der p* I has a molecular mass of 25 kDa, however under reducing and heating conditions *Der p* I appears to have a molecular mass of 30 kDa [13]. The characteristic molecular mass shift of *Der p* I was used to distinguish between the mite glutathione *S*-transferase allergen and *Der p* I on Western blots of mite proteins. The Coomassie Blue stained patterns of electrophoretically separated mite protein treatments 1, 2 and 3 can be seen in Fig. 3b, c and d, respectively. Western blots of the three different treatments of mite extracts were probed with sera that recognized the mite glutathione *S*-transferase and also bound to a 25-kDa protein corresponding to *Der p* I and the results for one of the sera are shown in Fig. 3. With treatment 1, binding to the 25 kDa was observed (Fig. 3e), with treatment 2 binding to both the 25- and 26-kDa proteins was observed (Fig. 3f), and with treatment 3 binding to the 26-kDa protein was observed and binding to the 25-kDa

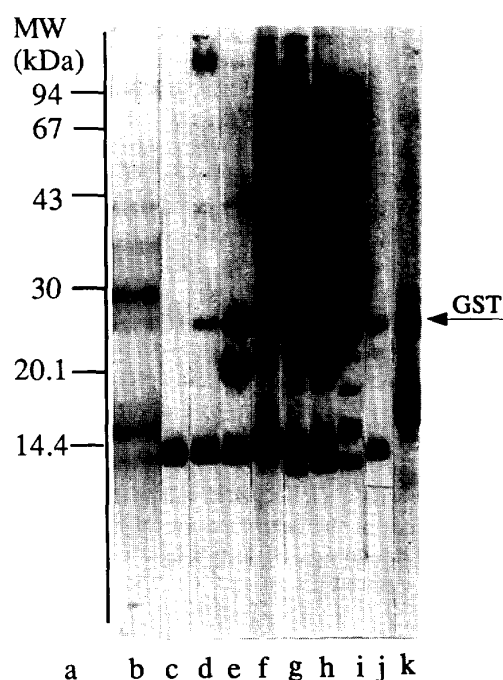


Fig. 2. Immunoblots showing the position of the 26-kDa mite glutathione *S*-transferase allergen. (a) Molecular weight markers; (b) Coomassie Blue-stained 9–27% gradient gel of mite extract (treatment 3, Materials and methods); (c) immunoblot of non-reduced mite probed with serum D; (d–k) immunoblots of heated and reduced mite extract probed with sera D–K from Table 1.

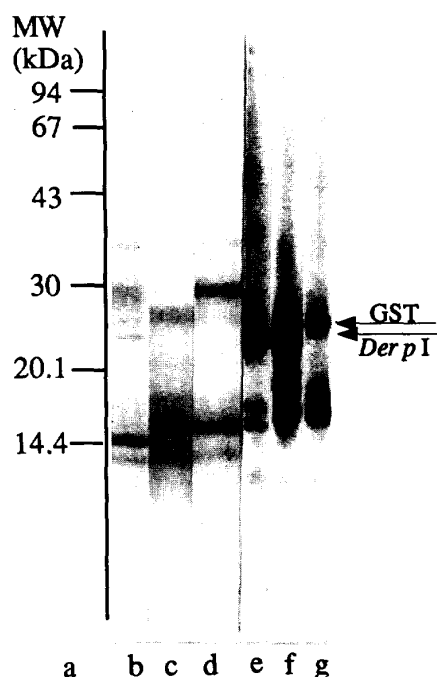


Fig. 3. Distinguishing *Der p* I from the mite glutathione *S*-transferase allergen on immunoblots of mite extract. (a) Molecular weight markers. Strips b–d: Coomassie Blue-stained gels of heated mite extract (b), reduced mite extract (c), and heated and reduced mite extract (treatments 1, 2 and 3, respectively, Materials and methods). Strips e–g: immunoblots of heated mite extract (e), reduced mite extract (f), and heated and reduced mite extract (g) probed with serum K.

protein was not observed (Fig. 3g). The results demonstrated that *Der p* I and mite glutathione *S*-transferase are two distinct bands on Western blots of mite extract. The results also suggested that the apparent molecular mass shift of *Der p* I from 25 to 30 kDa requires both heating and reduction of the mite extract.

4. Discussion

The isolation of a λ gt11 IgE-binding *D. pteronyssinus* cDNA clone led to the identification of a major new mite allergen that appears to be a mite glutathione *S*-transferase. IgE radioimmunoassays of mite allergic sera against the cloned fusion polypeptide revealed that the mite glutathione *S*-transferase is recognized by 40% of sera from mite allergic patients and of these sera more than 50% have a percentage uptake greater than 10 times the normal control. The presence of a major IgE-binding component of molecular mass 26 kDa, distinct from *Der p* I, has been previously reported [4]. The allergen was reported to be one of the nine polypeptides

that bound IgE antibodies most strongly and showed the highest frequency of binding to a large panel of sera, with a recognition frequency of approximately 50%. It would appear that the mite glutathione *S*-transferase allergen described in this paper corresponds to the frequently recognized 26-kDa allergen previously reported.

Attempts to identify the allergen in native mite extracts proved difficult using previously suggested non-reducing conditions for electrophoresis of mite proteins. Clear IgE binding to the 26-kDa allergen on Western blots of native mite extract could only be discerned under conditions of heating and reduction. The small difference in molecular mass between the 26-kDa allergen and *Der p* I suggested that the separation and identification of these two allergens by electrophoresis would be difficult. However, the reducing conditions necessary for the visualization of IgE-binding to the 26-kDa allergen also results in the apparent molecular mass of *Der p* I shifting from 25 to ~30 kDa [13]. It may be that the reduction of *Der p* I with its concurrent shift in molecular mass means that it is easier to distinguish IgE antibodies binding to the 26-kDa allergen on blots of heated and reduced mite proteins, or alternatively, the IgE-binding determinant/s of the 26-kDa allergen are only accessible on Western blots under reduced conditions.

IgE antibodies affinity-purified with the recombinant polypeptide were used to probe Western blots of reduced and heated mite extracts and the antibodies bound to a polypeptide of 26 kDa [10]. This polypeptide directly corresponded to the 26-kDa polypeptide identified through blotting studies with sera reactive to the cloned polypeptide. This then provided further evidence that the 26-kDa polypeptide observed on Western blots of mites was the same polypeptide as that encoded by the clone.

Three of the cloned mite allergens have similar molecular masses: *Der p* I [5], the 26-kDa glutathione *S*-transferase and the 22-kDa allergen which is recognized by cross-reacting antibodies that bind proteins of 24, 27 and 29 kDa on a Western blot [8], and there are a number of other minor allergens also of a close molecular mass [4]. The large number of mite IgE-binding polypeptides with a similar molecular mass emphasizes the importance of the determination of the nucleotide and derived amino acid sequence of each of the mite allergens to ensure their correct identification.

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