

Identification of a new major allergen of 39 kilodaltons of the storage mite *Lepidoglyphus destructor*

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(Received 23 October 1990; accepted 26 October 1990)

1. Summary

The allergen composition of a non-denatured extract of the storage mite *Lepidoglyphus destructor* was studied by a combination of hybridoma technology, sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and a sandwich radioallergosorbent test (four-step RAST).

Using hybridoma technology, monoclonal antibodies (mAbs) were generated against the non denatured extract of the *L. destructor*. After screening by ELISA, mAb 42B6 was selected for further studies. This mAb specifically recognized an antigen of *L. destructor* of 39 kDa in SDS-PAGE.

By a sandwich RAST, sera from 8 patients with known allergy to *L. destructor* were compared with control sera from 8 allergic patients. The results showed the presence of specific IgE against the 39-kDa protein in the sera of the test group, but not in the control group. There was also a good correlation observed between these data and the results obtained with the classical RAST.

These results indicate that the newly identified antigen of 39 kDa is a major allergen of the storage mite *Lepidoglyphus destructor*.

2. Introduction

The presence of several species of storage mites in farming environments has been well documented. Sites of predilection are storage barns, where mites are found in hay and buildings containing straw and grain dust [1–7]. Some species are also found in house dust [8–9]. Several studies have reported the importance of the storage mites as a cause of allergic disorders [6, 10–14]. However, there are only a few publications dealing with the identification of the responsible allergens [15, 16].

In a previous study, the allergen composition of the mite, *L. destructor* was identified by immunoblot analysis [17]. In the present study we identified a new allergen of the *L. destructor* that cannot be detected in Western blot. The antigenicity of the protein seems to be very sensitive to denaturing conditions where it loses its binding capacity to specific patient sera [17] and the mAb 42B6 produced against it.

3. Materials and Methods

3.1. Materials

Lepidoglyphus destructor extracts were prepared from raw material obtained from Allergon AB (Ängelholm, Sweden). Extraction was performed in 0.15 M NaCl as 1/10 (w/v), for 18 h at room temperature. The extracts were then centrifuged at 6600 × g for 10 min, filtered through a 0.8-μm Millex-HA filter (Millipore, Bedford, U.S.A.), desalted on a PD10 Sephadex column (Pharmacia, Uppsala,

Key words: *Lepidoglyphus destructor*; 39-kilodalton Allergen; Storage mite; Radioallergosorbent test, sandwich, four-step

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Sweden) and lyophilized. All extracts were stored at -20°C until used.

Sodium ^{125}I iodide was obtained from Amersham (Amersham, Bucks, U.K.). Monoclonal antibodies were purified from the hybridoma supernatants using protein G-Sepharose (Pharmacia). All other chemicals and reagents used were of analytical grade.

3.2. Production and screening of mAbs

Monoclonal antibodies against the *L. destructor* extract were produced using a standard hybridoma technique [18, 19]. The myeloma cell line Sp2.0 was used as cell fusion partner. After selecting the cultures for two weeks under HAT medium (6 mM Hypoxanthine, 50 mM aminopterin and 2 mM thymidine), hybridoma supernatants were screened for specific reactivity to the *L. destructor* extract using a standard enzyme-linked immunosorbent assay (ELISA) [20]. Antibodies reacting against *L. destructor* extract were selected, and the isotypes of the mAbs were determined by ELISA (ICN Biomedical Incorporated, IL, U.S.A.).

3.3. Radiolabelling of *L. destructor* proteins

1 mg of *L. destructor* extract was iodinated with 1 mCi of sodium ^{125}I iodide, using the commercial solid phase radioiodination reagent 1,3,4,6-tetrachloro-3 α ,6 α -diphenyl glycouril (Iodo-Gen) (Pierce Chemical Company, Rockford, IL, U.S.A.) according to the manufacturer's instructions.

3.4. Immunoprecipitation and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The anti-*L. destructor* (42B6) and immunoprecipitation-control monoclonal antibody (1B3, a monoclonal antibody against human p56^{lck} [21]) were each incubated with 100 μl of protein G-Sepharose equilibrated in PBS. After 1 h the antibody-bound protein G-Sepharose beads were washed 3 times with PBS. Samples were then incubated for 1 h under continuous mixing with 10×10^6 cpm of preabsorbed radiolabeled *L. destructor* extract. The immunocomplex-bound protein G-Sepharose beads were washed 6 times with

PBS, and then 60 μl of sample buffer was added. After boiling the samples for 3 min, the protein-containing sample buffers were analyzed using 10% SDS-PAGE. Pre-stained BioRad (BioRad, Richmond, CA, U.S.A.) molecular weight markers were used as SDS-PAGE standards. Gels were dried and autoradiographed on Amersham film with Agfa-60 intensifying screens (Agfa-Gevaert, F.R.G.) at -70°C .

3.5. Radioallergosorbent test (RAST)

Two variants of the radioallergosorbent test were performed: (1) the classical radioallergosorbent test (a three-step RAST, Phadebas RAST, disc d71), as previously described [10, 22], and (2) a sandwich RAST, a four-step modified version of the classical RAST. The classical RAST was modified for measurement of specific IgE antibodies in serum against the *L. destructor* proteins recognized by our monoclonal antibody 42B6. First, the monoclonal antibody 42B6 was coupled to cyanogen-bromide (CNBr)-activated paper discs [23]. After blocking the remaining active sites on the discs, they were successively incubated with (1) *L. destructor* extract or other extracts (negative control); (2) sera from patients either with or without (control) specific IgE antibodies to *L. destructor*; (3) ^{125}I -labeled immunosorbent-purified anti-IgE (Phadebas RAST, Pharmacia) of mixed D₁ and D₂ specificity [24].

4. Results

We have generated a battery of monoclonal antibodies against the non-denatured extract of *L. destructor*. The specificity of these antibodies was first screened by ELISA against the *L. destructor* extract and the positive ones were then also screened by ELISA against the culture medium used for growing the mite. The monoclonal antibody that exhibited the best reaction with the *L. destructor* extract but not the culture medium was selected for further studies. The isotype of the mAb 42B6 was determined to be IgG₃ using an ELISA technique.

To define which structure of *L. destructor* was recognized by the mAb anti-*L. destructor* (mAb 42B6), the extract was radiolabeled followed by immunoprecipitation with mAb 42B6. Fig. 1 shows the

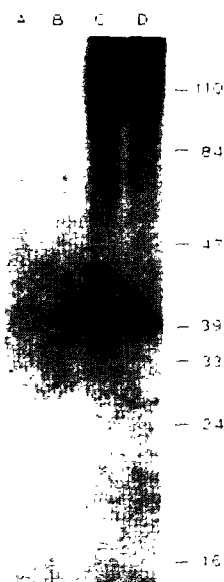


Fig. 1. SDS-PAGE analysis of the immunoprecipitation of the ^{125} I-labeled non-denatured *L. destructor* extract with non-specific mAb 1B3 (negative control) under non-reducing (lane A) and reducing (lane B) conditions, and mAb 42B6 (anti-*L. destructor*) under non-reducing (lane C) and reducing conditions (lane D). Molecular weight standards are expressed in kDa.

immunoprecipitation pattern of the mAb 42B6. This antibody immunoprecipitates a specific band with a relative molecular weight of 39000 under non-reducing conditions (lane C) as well as under reduced conditions (lane D). The immunoprecipitation controls with mAb 1B3 [21] did not show any band (lanes A and B). The 39-kDa protein failed to react with mAb 42B6 using Western blot technique (data not shown). Interestingly, sera from patients (1–8) positive in Fig. 2 were also negative in Western blot [17].

To determine the importance of the structure recognized by the mAb 42B6 in the atopic allergy caused by *L. destructor*, a sandwich RAST was established. In Fig. 2 sera from known *L. destructor*-allergic patients (sera 1–8) are compared with sera from 8 known non-*L. destructor*-allergic patients (sera 9–16). Sera 1–8 contained specific IgE antibodies against the structure recognized by the mAb 42B6. There was a good correlation with data obtained using classical RAST. The same antibody-coated discs and sera were used, but without the presence of *L. destructor* extract, as controls for the

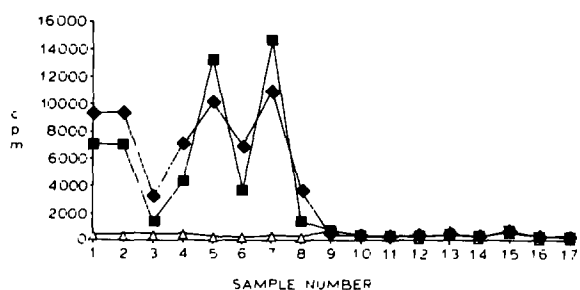


Fig. 2. Graphic presentation of the cpm values obtained in: (1) a sandwich four-step RAST with *L. destructor* extract (■); (2) a sandwich RAST without *L. destructor* extract (negative control) (△); and (3) a classical RAST (◆). Samples 1–8 are sera from patients known to be allergic to *L. destructor*. Samples 9–16 are control sera from patients allergic to allergens other than *L. destructor*. Sample 17 is a control without serum.

four-step RAST. A control without serum was also included (sample 17). None of the sera 9–16 showed any specific reactivity.

5. Discussion

Several studies have reported the importance of storage mites as a cause of allergic manifestations in people working in or living close to an agricultural environment [6, 10, 12–14]. In contrast to house dust mites [25, 26], only few antigens and allergens of the storage mites have been identified and characterized [15–17].

In a previous study, in the extract of the storage mite *L. destructor*, the major allergen components of 16–18 kDa were identified by immunoblot analysis [17]. In the present study we identified a new major allergen component of *L. destructor* apparently impossible to detect by Western blot technique. Its antigenicity seems to be affected by the manipulations preceding the Western blot analysis.

To study the antigen and allergen composition of *L. destructor*, mAbs against the non-denatured extract of the mite were produced. One of these monoclonal antibodies, mAb 42B6, specifically recognized a structure with an apparent molecular weight of 39000, under both reduced and non-reduced conditions.

To determine if the 39-kDa antigen is an allergen, we established a four-step sandwich RAST in which the antigenicity of the molecule is not affected. By this technique we have found high titers of specific

IgE antibodies against the 39-kDa antigen in sera from patients with known allergy to *L. destructor*. Since the exact concentration of antigen bound to the discs was not known, the cpm obtained from the four-step RAST cannot be used as absolute values to compare with the cpm obtained in the classical three-step RAST. However, it is clearly shown in the four-step RAST that there exist specific IgE antibodies in *L. destructor*-allergic individuals against the 39-kDa protein, as well as a good agreement with the data obtained by the classical RAST.

In conclusion, by combination of hybridoma technology, SDS-PAGE analysis and a sandwich RAST, we have identified a new major allergen of an apparent molecular weight of 39000 from the non-denatured extract of the storage mite, *L. destructor*.

Acknowledgements

This work was supported by grants from the Swedish Institute, the Karolinska Institute, the Swedish Medical Research Council, the Swedish Work Environment Fund (project No. 83-1182) and the Kon-sul Tore Nilsson Foundation.

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