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IgE and Monoclonal Antibody Reactivities to the Major Shrimp Allergen Pen a 1 (Tropomyosin) and Vertebrate Tropomyosins

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1. ABSTRACT

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1. ABSTRACT

Pen a 1, the major shrimp allergen from brown shrimp Penaeus aztecus has been identified as the muscle protein tropomyosin. To identify Pen a 1 IgE binding sites, the reactivities of Pen a 1-specific monoclonal antibodies (mAbs) and shrimp-allergic subjects' IgE to shrimp and homologous mammalian tropomyosins were analyzed. Pen a 1, purified by preparative SDS-PAGE and commercially obtained porcine, bovine and rabbit tropomyosin were cleaved by CNBr or digested by endoproteinases Lys-C, Glu-C, trypsin, Arg-C and chymotrypsin. Reactivities of Pen a 1-specific mAbs and IgE to the resulting peptides were analyzed by dot blot and immunoblotting. The dot blot analysis showed that mAbs and IgE antibodies did not react with any of the mammalian tropomyosins. The immunoblot analysis showed that all Pen a 1 digests bound IgE or mAbs. However, not all peptides in each digest possessed an IgE binding site. IgE binding intensity and frequency varied by subject and peptide digest. IgE and mAb reactivity patterns were similar but no mAb reproduced the IgE binding patterns indicating that subjects' IgE bound some epitopes that were not recognized by the Pen a 1-specific mAbs. These studies suggest that IgE-binding epitopes are restricted to certain parts of the Pen a 1 molecule, Pen a 1 may have several similar epitopes, and that Pen a 1 epitopes do not appear to be located in the highly homologous parts of the tropomyosin molecule.

2. INTRODUCTION

At least 13 IgE-binding bands had been identified in the extract of brown shrimp (Penaeus aztecus). Eighty-two percent of subjects' sera bound to a 36 kD protein, designated as Pen a 1, the only major allergen in shrimp [1]. Pen a 1-like bands were also detected in other Crustacea such as crawfish (Procambarus clarkii), crab (Callinectes sapidus) and lobster (Panulirus argus) suggesting a common Crustacea allergen [2, 3]. Pen a 1 was identified as the muscle protein tropomyosin by sequencing a 21 amino acid residue peptide obtained by endoproteinase digestion and HPLC purification [1, 4]. This amino acid sequence showed a 60 to 87% sequence homology

with tropomyosins ranging from human to fruitfly tropomyosin [4, 5]. The aim of this study was the immunoblot. analysis of IgE and Pen a 1-specific mAb reactivities to Pen a 1, homologous vertebrate tropomyosins and their fragments. For these purposes the major shrimp allergen Pen a 1 was purified by preparative SDS-PAGE and purified Pen a 1, chicken, rabbit, beef and porcine tropomyosin were cleaved with CNBr and endoproteinases. The resulting peptide-containing digests were analyzed by immunoblot and dot blot of digests with patients' IgE and Pen a 1-specific mAbs.

3. MATERIAL AND METHODS

3.1. Subjects' sera

Sera were collected from atopic shrimp-sensitive individuals, with histories of respiratory, dermatologic, or gastrointestinal symptoms occurring within one hour of shrimp ingestion. All individuals were skin test positive (wheal $\geq$3 mm) to shrimp and demonstrated elevated levels of IgE antibodies (RAST binding $\geq$3%) to shrimp extract.

3.2. Purification of Pen a 1

Pen a 1 was purified by preparative SDS-PAGE (Model 491 PrepCell, Biorad). Shrimp extract [6] was separated on the 28 mm ID column using Laemmli's discontinuous SDS-PAGE buffer system [7]. A 15 mm-high stacking gel (5%T, 1.5%C) was poured on top of the 60 mm-high separation gel (10%T 1.5%C). The fractions were analyzed by SDS-PAGE and immunoblotting using a serum pool of shrimp-allergic subjects and Pen a 1-specific (mAbs). Fractions that contained pure Pen a 1 were pooled, dialyzed and lyophilized.

3.3. Production of monoclonal antibodies (mAbs)

BALB/c mice were immunized twice at days 1 and 7 with 5 μg of purified Pen a 1. After five weeks, spleen cells were fused with P3X63Ag8.U1 myeloma cells (ATCC CRL 1597) using PEG fusion technique [8, 9]. Cells were screened for antibody-producing hybridomas by ELISA, checked for specificity by immunoblotting and cloned by limiting dilution.

3.4. Peptide Production

100 µg purified Pen a 1 or tropomyosin from beef, pork, rabbit and chicken (Sigma) were incubated overnight in the dark at room temperature in 10% CNBr in 70% formic acid [10]. CNBr products were lyophilized. For endoproteinase digestion, 100 µg tropomyosin were denatured in 50 µl denaturation buffer (50 mM Tris/HCl pH 8.0, 6 M urea, 5 mM DTT) followed by 1:8 dilution in protease buffer (Lys-C: 25 mM Tris/HCl pH 7.7, 1 mM EDTA, Glu-C I: 50 mM NH_4CH_3COO/CH_3COOH pH 4.0; Glu-C II: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.8; trypsin: 50 mM Tris/HCl pH 7.6, 1 mM $CaCl_2$; alkaline protease: 50 mM Tris/HCl pH 9.0, 5 mM $CaCl_2$; Arg-C: 50 mM Tris/HCl pH 7.5, 1 mM $CaCl_2$, 2 mM DTT; chymotrypsin: 50 mM Na_2HPO_4/NaH_2PO_4 pH 7.0). One µg of enzyme was added and Pen a 1 was digested overnight at 37°C.

3.5. Peptide SDS-PAGE and Immunoblotting

The buffer system of Schägger and von Jagow [11] with slight modifications was used. A 10 mm-high stacking gel (5%T, 2.5%C) was poured on top of the 60 mm-high separation gel (17.5%T, 2.5%C). The cleavage products were diluted in sample buffer and boiled for 5 min. The separated peptides were electrophoretically transferred [12] onto CNBr-activated nitrocellulose membranes [13]. The individual IgE and mAb reactivities were detected using alkaline phosphatase-labeled, monoclonal anti-human-IgE (Southern Biotechnology Associates) and goat anti-mouse-IgG+IgM (Jackson Immunoresearch Laboratories) in combination with the chemiluminescence substrate CSPD (Tropix).

3.6. Dot Test

1 µl of tropomyosins (1 µg/µl) and their digests (1 µg/µl) were applied onto CNBr-activated nitrocellulose membrane. After air-drying and blocking with TBS-Tween, the dot blots were incubated in diluted patients' serum pool (1:100) and mAbs (1:2), respectively. The IgE and mAb reactivities were detected as described above.

4. RESULTS

4.1. Shrimp tropomyosin Pen a 1

All cleavage procedures, with the exception of alkaline protease digestion, resulted in IgE-binding peptides. Not all Pen a 1 fragments bound IgE or mAbs. Seven/8 shrimp-allergic subjects reacted with Pen a 1 peptides. The IgE binding intensity and frequency varied by subject and peptide digest. For example, Arg-C fragments bound IgE of 6 subjects whereas trypsin fragments were detected by only 3 subjects' IgE; individual subjects reacted up to 7/8 Pen a 1 digests (Table 1). Depending on the digest, the IgE-binding peptides had a molecular weight as high as 20 kD and as low as 4 kD. Six/9 of the Pen a 1-specific mAbs reacted with Pen a 1 peptides and all 6 to IgE-binding peptides. Six/8 cleavage procedures (CNBr, Lys-C, Glu-C I, Glu-C II, trypsin, Arg-C) produced both IgE and mAb binding peptides whereas the digestion with chymotrypsin produced only IgE binding peptides. IgE and mAb reactivities showed similar but not identical binding patterns (figure 1). An 8 kD CNBr peptide was detected by both mAb 8 and IgE whereas a 14 kD peptide was only detected by mAb 8. The mAbs 1 to 4 showed their strongest reactivities to an 18 kD Lys-C fragment whereas the most intense IgE binding occurred to 10 to 18 kD fragments.

4.2. Beef, Pork, Rabbit and Chicken Tropomyosin

Pen a 1-specific mAbs and Pen a 1-specific IgE of shrimp-allergic subjects did not bind to any of the vertebrate tropomyosins both by dot blot and immunoblot analysis (Fig. 2).

5. DISCUSSION

Immunologically active, IgE-binding Pen a 1, purified by preparative SDS-PAGE, was cleaved by CNBr and endoproteinases Lys-C, Glu-C, trypsin, alkaline protease, Arg-C and chymotrypsin. With the exception of alkaline protease digestion, all cleavage procedures resulted in IgE-binding peptides. Not all peptides generated bound IgE. indicating that IgE-binding epitopes are restricted to certain parts of Pen a 1. cleavage by CNBr, Lys-C, trypsin, Glu-C and Arg-C resulted in mAb-binding peptides. In contrast to IgE reactivities, chymotrypsin fragments were not detected by any mAb. Since the same mAb reacted to different peptides from the same digest, Pen a 1 may have

several similar epitopes. The comparison of IgE and mAb reactivities demonstrated similar but not identical binding, indicating that these Pen a 1-specific mAbs and subjects' IgE bound to different epitopes. Pen a 1-specific mAbs and IgE did not bind highly homologous vertevtate tropomyosins from chicken, rabbit, beef and porc indicating that IgE and mAb binding epitopes may be restricted to the variable parts of Pen a 1. Further sequence analysis and epitope mapping of Pen a 1 and other tropomyosins will advance our understanding of the molecular basis and pathogenesis of shrimp allergy in particular and food allergy in general.

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6. REFERENCES

1 Daul CB, Slattery M, Reese G, Lehrer SB: Identification of the major brown shrimp (Penaeus aztecus) as the muscle protein tropomyosin. Int Arch Allergy Clin Immunol 1994;105:49-55.

2 Daul CB, Slattery M, Morgan JE, Lehrer SB: Identification of a common major crustacea allergen. J Allergy Clin Immunol 1992;89:194.

3 Halmepuro L, Salvaggio JE, Lehrer SB: Crawfish and lobster allergens: Identification and structural similarities with other crustacea. Int Arch Allergy Appl Immunol 1987;84:165-72.

4 Daul CB, Slattery M, Morgan JE, Lehrer SB: Common crustacea allergens: identification of B cell epitopes with shrimp-specific monoclonal antibodies; in Kraft D, Sehon A (eds): Molecular Biology and Immunology of Allergens. Boca Raton, Ann Arbor, London, Tokyo, CRC Press, 1993, pp 291-294.

5 Daul CB, Slattery M, Lehrer SB: Shared antigenic/allergenic epitopes between shrimp Pen a I and fruit fly extract. J Allergy Clin Immunol 1993;91:341.

6 Daul CB, Morgan JE, Waring NP, McCants ML, Hughes J, Lehrer SB: Immunologic evaluation of shrimp-allergic individuals. J Allergy Clin Immunol 1987;80:716-721.

7 Laemmli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970;227:680-685.

8 Goding JW: Antibody production by hybridomas. J Immunol Methods 1980;39:285-308.

9 Köhler G: The technique of hybridoma production; in Lefkovits, Pernis (eds): Immunological Methods, Academic Press, New York, 1981, vol 2, pp 285-298.

10 Gross E: The cyanogen bromide reaction. Methods Enzymol 1967;11:238-255.

11 Schägger H, von Jagow G: Tricine-sodium dodecyl-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem 1987;166:368-379.

12 Kyhse-Andersen J: Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. J Biochem Biophys Methods 1984;10:203-209.

13 Demeulemester C, Peltre G, Laurent M, Pankeleux D, David B: Cyanogen bromide-activated nitrocellulose membranes: A new tool for immunoprint techniques. Electrophoresis 1987;8:71-73.

Legends for Figures and Tables

Figure 1: Comparison of IgE and mAb reactivities to CNBr and Lys-C cleavage-derived Pen a 1 fragments.

Figure 2: IgE and mAb reactivities to Lys-C peptides of shrimp (Pen a 1), chicken, rabbit, porcine and beef tropomyosins (Tm's).

Table 1: Comparison of IgE and mAb reactivities to Pen a 1 fragments obtained by CNBr cleavage and endoproteinase (Lys-C, Glu-C, trypsin, alkaline protease, Arg-C, chymotrypsin) digestion. MAb reactivities representing mAb binding with IgE-binding Pen a 1 peptides are blocked.