**COMMON CRUSTACEA ALLERGENS: IDENTIFICATION OF B CELL EPITOPES WITH THE SHRIMP SPECIFIC MONOCLONAL ANTIBODIES

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I. INTRODUCTION

Seafood is a very popular food source throughout the world and most information indicates that it will continue as a major component of our diet. However, adverse reactions to seafood products can and do occur. Some seafoods contain normal ordinarily harmless protein molecules which can cause life threatening allergic reactions in sensitive individuals.[1]

Crustacea are a frequently eaten seafood in New Orleans and throughout the Gulf South region of the United States. Since allergic reactions to crustacea are often observed in our clinical practice, we chose to investigate shrimp allergy as a model of seafood allergy.[2] Our previous studies of allergic reactions to shrimp indicate that the majority are caused by IgE antibody reactivity with shrimp allergens.[2] A number of allergens were demonstrated in crude shrimp extracts.[3,4] Several major shrimp allergens cross-react with those present in crawfish, lobster, and crab suggesting that crustacea contain common allergens.[4,5]

Studies from our laboratory suggest that the 36 kD protein from the shrimp Penaeus aztecus (Pen a bd36K) is a major shrimp allergen.[6] SDS-PAGE/Western Blot analyses indicate that at least 86% of shrimp sensitive subjects tested react to this allergen.[7] Purified 36 kD shrimp allergen can inhibit 85% of the RAST to crude shrimp extract. This allergen is an acidic protein with an isoelectric point of 5.2. The current investigations were undertaken to further characterize Pen a bd36K by using monoclonal antibodies (mAbs) to study its epitope structure in relationship to other crustacea allergens.

II. MATERIAL AND METHODS

Shrimp allergen extracts were prepared by homogenation of P. aztecus shrimp meat in phosphate buffered saline as described.[2] A 36 kD shrimp allergen was purified following SDS polyacrylamide gel electrophoresis by electroelution.[6] Because the amino terminus was blocked, Pen a bd36K was digested with endoproteinase lys-C and a 21 residue peptide was isolated by HPLC.[8] The amino acid sequence was determined by the W.M. Keck Foundation Biotechnology Resource Laboratory at Yale University, New Haven, CT according to established procedures.[8]

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Balb/c mice were immunized with P. aztecus shrimp extract and boosted with Pen a bd36K. Spleen cells were fused with P3X-63-Ag.8.653 mouse myeloma cells.[9] Resultant fusion products were screened for ELISA reactivity against shrimp extract. Hybridomas demonstrating shrimp specific antibody were subcloned and mAbs purified from culture supernatants by 80% ammonium sulfate precipitation. Shrimp proteins separated by SDS-PAGE electrophoresis were transferred to nitrocellulose membrane.[9] Strips were incubated sequentially with mouse hybridoma supernatant followed by I^{125} labelled anti-mouse IgG. After the final wash, strips were placed on x-ray film and audioradiography performed for 24 to 48 hours.

III. RESULTS

The amino acid sequence of the 21 residue peptide from Pen a bd36K is shown in Figure 1. Over 2-1/2 million proteins in the Swiss protein database were compared for homology with the amino acid sequence of this peptide. Results indicate significant (60-87%) homology with the muscle protein tropomyosin. The greatest homology (72-87%) was observed with tropomyosin of the fruit fly, another member of the phylum Arthropoda. Less (60-62%), although still significant, homology was observed with tropomyosin from human, chicken, rat, and mouse.

VAL	LEU	GLU	ASN	ARG	SER	LEU	SER	ASP	GLU	GLU
1	2	3	4	5	6	7	8	9	10	11

ARG	MET	ASP	ALA	LEU	GLU	ASN	GLN	LEU	LYS
12	13	14	15	16	17	18	19	20	21

Figure 1 - Amino Acid Sequence of a 21 Residue Peptide from Pen a bd36K

Nine clones exhibited anti-shrimp activity by ELISA; all 9 mAbs reacted with multiple shrimp protein bands by SDS-PAGE/Western Blot analysis. The reactivity of 6 mAbs to P. aztecus is shown in Figure 2.

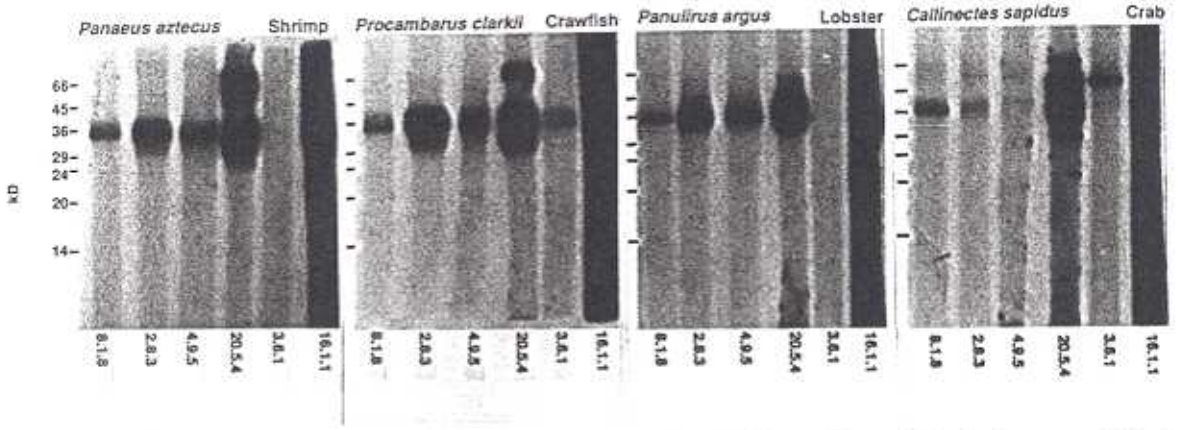

Figure 2 - Monoclonal Antibody Reactivity with Shrimp, Crawfish, Lobster and Crab Proteins

Five/6 mAb had significant reactivity to the 36 kD shrimp allergen, as well as to shrimp proteins at 29, 30, 45 and 66 kD. All mAbs recognized multiple protein bands with pI's 4.5 to 6.0 as determined by IEF/immunoprint studies. Reactivity of shrimp mAbs with other crustacea was also analyzed (Figure 2). The major 36 kD antigen was identified in crawfish (Procambarus clarkii), crab (Callinetes sapidus) and lobster (Panularus argus). The patterns of reactivity were not unlike those observed with shrimp extracts. The mAbs also reacted to epitopes in several other bands of these crustacea extracts.

IV. DISCUSSION

These studies indicate that a 36 kD acidic protein which is substantially similar to the 38.3 kD shrimp allergen isolated by Hoffman[10] and the 34 kD allergen described by Nagpal[11] is a major shrimp allergen. The amino acid sequence of a 21 residue peptide obtained from this protein has significant homology with the muscle protein tropomyosin. MAb reactivity indicates that epitopes of Pen a bd36K are detected in other shrimp proteins as well as proteins present in crawfish, lobster, and crab. The fact that these crustacea extracts contain proteins of similar molecular weight and structure, supports the hypothesis that common major crustacea allergens exist. Eventually, these results may be employed for development of diagnostic, as well as therapeutic, reagents for the treatment of crustacea allergy.

V. REFERENCES

1. Taylor, S.L., Bush, R.K., in Vol. 3, Handbook of Natural Toxins, 1988.
2. Daul, C.B., Morgan, J.E., Waring, N.P., McCants, M.L., Hughes, J., Lehrer, S.B., JACI, 80:716, 1987.
3. Lehrer, S.B., McCants, M.L., Salvaggio, J.E., Int. Archs Allergy Appl. Immun., 77:192, 1985.
4. Lehrer, S.B., Ann Allergy, 57:267, 1986.
5. Halmepuro, L., Salvaggio, J.E., Lehrer, S.B., Int. Archs Allergy Appl. Immunol 84:165, 1987.
6. Daul, C.B., Slattery, M., Morgan, J.E., Lehrer, S.B., (Abst) JACI 87:192, 1991.
7. Morgan J.E., Daul, C.B., Lehrer, S.B., English, M., (Abst) JACI, 85:170, 1990.
8. Stone, K., Lopesti, M., Crawford, M., DeAngelis, R., Williams, K., in A practical guide to protein and peptide purification for micro sequencing. Matsudaira, P., Ed., Academic Press, 1989, 31.
9. Daul, CB., Morgan, J.E., Lehrer, S.B., English, M. (Abst) JACI 85:276, 1990.
10. Hoffman, D.R., Day Jr., E.D., Miller, J.S., Ann Allergy, 47:17, 1981.
11. Nagpal, S., Metcalfe, D.D., Subba Rao, P.V., J Immunol, 138:4169, 1987.

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