

Source characterization and molecular structure of cockroach allergens

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SUMMARY

The application of molecular cloning techniques has led to the identification of important allergens from both German and American cockroach (*Blattella germanica* and *Periplaneta americana*). These allergens include Bla g 2 (aspartic protease) Bla g 4 (lipocalin) Bla g 5 (glutathione-S-transferase) and Per a 3 (hemocyanin). Only the Group 1 allergens Bla g 1 and Per a 1 appear to be expressed by both species. The Group 1 allergens are composed of 2-7 tandem amino acid repeats of ~100 amino acids and show homology to mosquito ANG12 precursor and mitochondrial energy transfer proteins. The prevalence of IgE responses to the cloned allergens ranges from 30-80 % and Bla g 2 Bla g 4 and Bla g 5 show strong skin test reactivity. High level expression systems have been developed to produce recombinant cockroach allergens in bacteria or yeast for research purposes and clinical studies. Use of the recombinant allergens will enable antibody and T cell epitopes to be identified and will allow new strategies for cockroach immunotherapy to be developed.

KEY-WORDS: Cockroaches. - Allergy.

INTRODUCTION

Epidemiologic studies have established that cockroach (CR) allergens are an important cause of asthma. In inner city areas of the US, up to 60 % of asthma patients are sensitized to CR allergens and CR sensitization is a risk factor for admission

RÉSUMÉ

Caractérisation de la provenance et structure moléculaire des allergènes de la blatte. – L'utilisation des techniques de clonage moléculaire a conduit à l'identification d'importants allergènes provenant des blattes germaniques et américaines (*Blattella germanica* et *Periplaneta americana*). Ces allergènes comprennent Bla g 2 (protéase aspartique), Bla g 4 (lipocalin) Bla g 5 (glutathion-S-transférase) et Per a 3 (hémocyanine). Seuls les allergènes du Groupe 1, Bla g 1 et Per a 1 paraissent être exprimés dans les deux espèces. Les allergènes du Groupe 1 sont composés de la répétition d'un amino-acide en 2-7 tandem de ± 100 amino-acides et présente une homologie avec le pré-curseur ANG 12 du moustique et les protéines de transfert d'énergie des mitochondries. La prévalence des réponses IgE aux allergènes clonés va de 30 à 80 % et les tests cutanés avec Bla g 2, Bla g 4 et Bla g 5 révèlent une forte réactivité. Des systèmes d'expression de haut niveau ont été développés pour créer des allergènes recombinants de blattes dans des bactéries ou des levures en vue de recherches et d'études cliniques. L'usage de ces allergènes recombinants permettra d'identifier les épitopes d'anticorps et de lymphocytes T et de développer de nouvelles stratégies pour l'immunothérapie des blattes.

MOTS-CLÉS: Blattes.- Allergie.

to hospital Emergency Rooms (reviewed in [1]). Asthma mortality and morbidity is increasing in the US, especially among lower socio-economic groups, where CR allergy is a particular problem. A recent study has shown that sensitization and exposure to CR allergens was associated with increased hospitalization rates and asthma morbi-

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dity among inner city children in the US [2]. Cockroach allergy is also prevalent in other parts of the world, including Japan, Taiwan and Brazil. Cockroach induced asthma is associated with high levels of CR infestation in low cost public housing in Strasbourg and one study estimated that CR allergy could affect ~5 % of the French population [3].

The principal domiciliary CR species are *Blattella germanica* and *Periplaneta americana*. Over the past five years great progress has been made towards defining CR allergens using molecular biology techniques. Screening *B. germanica* and *P. americana* cDNA libraries with human IgE antibodies or allergen specific murine monoclonal antibodies has resulted in the identification of panels of allergens from both species. These allergens are being produced in bacterial or yeast expression systems so that the recombinant allergens can be used for research, diagnosis and, ultimately, the treatment of CR allergy.

STRUCTURE AND FUNCTION

Blattella germanica allergens

A list of cloned *B. germanica* allergens, their molecular properties, GenBank accession num-

bers and WHO/IUIS nomenclature is shown in table I. Details of the cloning of Bla g 2, Bla g 4 and Bla g 5 have been published and sequence comparisons with have shown that the allergens belong to several protein families [4-5]. Bla g 2 shows up to 30 % homology with aspartic proteases such as pepsin human cathepsins and mosquito lysosomal protease [4].

Bla g 4 was the first *B. germanica* allergen to be cloned and is a member of the ligand binding protein family (also known as lipocalins or calycins). Interestingly, this family contains important animal allergens, including mouse and rat urinary allergens, and dog, cow and horse epithelial allergens. Lipocalins act to bind or transport small hydrophobic chemicals and have a diverse array of functions. The rodent urinary allergens are pheromone binding proteins and it is tempting to speculate that Bla g 4 may have a similar function in CR, a possibility worthy of further investigation. Although the overall sequence homology between lipocalins is only ~20 %, they have a conserved three dimensional structure and it was possible to model the structure of Bla g 4 based on its homology to butterfly bilin binding protein, which had been crystallized. The structure is a cup shaped, eight stranded anti-parallel β barrel, with three structurally conserved regions located at the base of the cup [5].

Table I. – Current nomenclature, structure and function of cockroach allergens.

Allergen	cDNA size (bp)	Amino Acids	Calculated MW (kDa)	Accession No.	Function
A: <i>Blattella germanica</i>					
Bla g 1.0101	1429	412	46	AF072219	Unknown
Bla g 1.02	1791	492	56	AF072220	–
Bla g 1.0102	4058	–	–	L47595	–
Bla g 2	1056	328	36	U28863	Aspartic protease
Bla g 4	546	182	21	U40767	Lipocalin (Calcycin)
Bla g 5	1140	203	23	U92412	Gluthione-S-Transferase
Bla g 6	582	38	21	NA	Troponin
B: <i>Periplaneta americana</i>					
Per a 1.0101	870	231	26	AF72222	Unknown
Per a 1.0102	890	220	25	U78970	–
Per a 1.0103	1432	395	45	U69957	–
Per a 1.0104	1024	274	31	U69261	–
Per a 1.02	1630	446	51	U69260	–
Per a 3.01	2418	669	79	L40818	Arylphorin/hemocyanin
Per a 3.0201	2274	631	75	L40820	Arylphorin/hemocyanin
Per a 3.0202	1410	470	56	L40819	Arylphorin/hemocyanin
Per a 3.0203	1179	393	47	L40821	Arylphorin/hemocyanin
Per a 7	1330	284	33	NA	Tropomyosin

Bla g 5 is a member of the GST-2 family of glutathione S-transferases and shows 40-50 % homology to other insect GST's, and 28 % homology to the house dust mite GST allergen (Der p 8) [6]. The function of the GST is thought to be in detoxification of foods ingested by CR and it is possible that GST allergen production could be upregulated by the use of insecticides.

By Northern analysis, it was not possible to detect mRNA for Bla g 2 or Bla g 4 in *P. americana* suggesting that these allergens were species specific. Similarly, *P. americana* extracts did not inhibit IgE ab binding to Bla g 5 in RIA. The only cross-reacting allergens that have so far been sequenced from both *B. germanica* and *P. americana* are the Group 1 allergens Bla g 1 and Per a 1. Bla g 1 cDNA clones were obtained by screening a *B. germanica* cDNA library with an anti-Bla g 1 mAb (10A6). The Bla g 1 allergen occurs as two isoforms showing 75 % amino acid sequence [7]. Bla g 1 also shows 94 % homology to a previously reported nucleotide sequence termed Bla g Bd90K [8]. A striking feature of the Group 1 CR allergens is that they consist of a number of tandem amino acid repeats of approximately 100 amino acid residues. The proteins contain trypsin cleavage sites and occur in multiple molecular sizes, depending upon the number of amino acid repeats they contain and the extent of trypsin cleavage that occurs in the CR digestive system [7].

Periplaneta americana allergens

The function of the Group 1 allergens is not clear, however, two Per a 1 isoforms have been cloned and the Per a 1.01 sequence occurs as four isoallergenic variants (table IB) [9, 10]. Sequence comparisons show that the Bla g 1 and Per a 1 allergens show homology to a mosquito protein ANG 12, which is thought to be involved in digestion of a bloodmeal [7, 9, 10]. The Group 1 CR allergens also contain motifs that are found in mitochondrial energy transfer proteins which also have three amino acid repeats similar to those seen in the Group 1 allergens.

Two other *P. americana* allergens have been cloned: Per a 3 which shows homology to insect storage proteins (arylephorins) or hemocyanins; and Per a 7, which is tropomyosin (table IB) [11, 12, 13]. Per a 3 has two isoforms Per a 3.01 (79kd) and Per a 3.02 (75kd), with 69 % homology and there are three variants of Per a 3.02. The variants appear to be truncated forms of Per a 3.02 containing central antigenic determinants of the molecule [12].

Table II. – Expressed recombinant cockroach allergens.

Allergen	Expression system	Vector	Yield (mg/L)
Cockroach			
Per a 1	<i>Pichia pastoris</i>	pPIC9	14
	<i>E. coli</i>	pET21b	18
Per a 3	<i>E. coli</i>	pET	–
Bla g 2	<i>Pichia pastoris</i>	pPIC9	~10
Bla g 4	<i>Pichia pastoris</i>	pPIC9	50
Bla g 5	<i>E. coli</i>	pPET21a	3-4

Table III. – Biologic activity of natural and recombinant cockroach allergens¹.

Patient	Intradermal skin test titer			
	<i>B. germanica</i>	nBla g 2	rBla g 4	rBla g 5
JS	10 ^{-5.5}	10 ⁻³	10 ⁻³	10 ⁻⁴
SE	10 ^{-5.5}	10 ^{-2.5}	10 ⁻⁵	10 ⁻³
TB	10 ⁻⁵	n.d.	10 ⁻⁵	10 ⁻³
RM	10 ^{-5.5}	10 ⁻³	10 ⁻³	10 ⁻¹
MB	10 ⁻⁶	10 ^{-2.5}	> 1	n.d.
ST	10 ^{-5.5}	10 ⁻¹	> 1	> 1
Controls (n= 4)	10 ⁻²	> 10	> 10	> 10

1. Intradermal skin test titers in six cockroach allergic patients and four non-allergic controls [4-6].

ALLERGENIC IMPORTANCE OF COCKROACH ALLERGENS

Skin tests and *in vitro* serologic assays have established that the prevalence of IgE ab to natural Bla g 1 and Bla g 2 ranges from 30-50 % and 60-80 % respectively. Recombinant CR allergens have been produced in *E. coli* Bla g 4, Bla g 5, Per a 1, Per a 3) or in the yeast *Pichia pastoris* (Per a 1, Bla g 2, Bla g 4) (table II) [14]. Serologic studies suggest that the prevalence of IgE ab to rBla g 4 and rBla g 5 is 60 % and 70 %, respectively and that a cocktail of Bla g 1, Bla g 2, Bla g 4 and Bla g 5 would diagnose 95 % of patients with CR allergy. Quantitative intradermal skin tests show that these allergens give positive reactions using pg amounts of allergen (down to 10⁻⁶ µg/ml) (table III).

For *P. americana* the prevalence of IgE ab to Per a 1 is 30-50 %. Skin tests using natural Per a 3 gave positive reactions in 83 % of CR allergic patients, however, only 47 % of patients showed reactivity to rPer a 3.01 and rPer a 3.02 expressed in the

pET system [12]. Marked differences in skin test reactivity have been observed between cloned Per a 3.0202 (26 %) and 3.0203 (94 %). Per a 7 has only recently been cloned and plaque immunoassay results suggest that approximately 70 % of CR allergic patients have IgE ab to this allergen. While further assessment of the allergenic reactivity of recombinant *P. Americana* allergens is required the current data leaves little doubt that Per a 1 Per a 3 and Per a 7 are important allergens that should be considered for research and clinical use.

CONCLUSIONS

Panels of important CR allergens have been cloned and produced as recombinant proteins in high level expression vectors. Several allergens appear to be enzymes produced in the CR digestive system. With the exception of the Group 1 allergens, CR allergens identified to date appear

to be species specific. Monoclonal antibodies have been raised against Bla g 1, Per a 1, Bla g 2 and Per a 3. These mAb have been used in immunoassays for standardization of CR extracts and for assessments of environmental allergen exposure (reviewed in [1]). These assays will enable clinically significant levels of CR allergen exposure to be established and the effects of insecticides on CR allergen levels to be investigated.

Expression of recombinant CR allergens will provide tools to determine the three dimensional structure of the allergens and the amino acid sequence data will enable strategies for the identification of T cell epitopes to be pursued. The sequences will also be available for use in trials of new forms of immunotherapy, e.g. plasmid DNA vaccines or use with other Th1 like adjuvants. The use of recombinant CR allergens should allow mechanisms of CR allergen induced asthma to be investigated and may lead to new approaches to asthma treatment in the future.

REFERENCES

1. Chapman M.D., Vailes L.D., Hayden M.L., Platts-Mills T.A.E., Arruda L.K. – Cockroach Allergens and their Role in Asthma. In : Kay A.B. – *Allergy and Allergic Disease*. Oxford, Blackwell Science Ltd., 1997.
2. Rosenstreich D.L., Eggleston P., Kattan M., Baker D., Slavin R.G., Gergen P., Mitchell H., Mc Niff-Mortimer K., Lynn H., Ownby D., Malveaux F. – The Role of Cockroach Allergy and Exposure to Cockroach Allergen in Causing Morbidity Among Inner-City Children with Asthma. *N. Engl. J. Med.*, 1997 ; 336 : 1356j-1363j.
3. De Blay F., Sanchez J., Hedelin G., Perez-Infante A., Verot A., Chapman M.D., Pauli G. – Dust and Airborne Exposure to Allergens Derived from Cockroach (*Blattella germanica*) in low-cost public housing in Strasbourg (France). *J. Allergy Clin. Immunol.*, 1997, 99, 107-112.
4. Arruda L.K., Vailes L.D., Mann B.J., Shannon J., Fox J.W., Vedvick T.S., Hayden M.L., Chapman M.D. – Molecular Cloning of a Major Cockroach (*Blattella germanica*) Allergen Bla g 2. *J. Biol. Chem.*, 1995, 270, 19563-19568.
5. Arruda L.K., Vailes L.D., Hayden M.L., Benjamin D. C., Chapman M.D. – Cloning of Cockroach Allergen Bla g 4, Identifies Ligand Binding Proteins (or Calycons) as a Cause of IgE Antibody Responses. *J. Biol. Chem.*, 1995, 270, 31196-31201.
6. Arruda L.K., Vailes L.D., Platts-Mills T.A.E., Hayden M.L., Chapman M.D. – Induction of IgE Antibody Responses by Glutathione S-Transferase from the German Cockroach (*Blattella germanica*). *J. Biol. Chem.*, 1997, 272, 20907-20912.
7. Pomes A., Melen E., Vailes L.D., Retief J.D., Arruda L.K., Chapman M.D. – Novel Allergen Structures with Tandem Amino Acid Repeats Derived from German and American Cockroach. *J. Biol. Chem.* 1998, 273, 30801-30807.
8. Helm R., Cockrell G., Stanley J.S., Brenner R.J., Burks W., Bannon GA. – Isolation and Characterization of a Clone Encoding a Major Allergen (Bla g Bd90K) involved in IgE-mediated cockroach hypersensitivity. *J. Allergy Clin. Immunol.*, 1996, 98, 172-180.
9. Wu C.H., Wang N.M., Lee M.F., Kao C.Y.Y., Luo S.F. – Cloning of the American Cockroach Cr-PII Allergens: Evidence for the Existence of Cross-reactive Allergens Between Species. *J. Allergy Clin. Immunol.*, 1998, 101, 832-840.
10. Melen E., Vailes L.D., Pomes A., Arruda K.L., Chapman M.D. – Molecular Cloning of Per a 1, an IgE Cross Reactive Allergen produced by American and German Cockroaches with homology to mosquito ANG12 Gene. 1998. *J. Allergy Clin. Immunol.*, 1999 (In press).
11. Wu C.H., Lee M.F., Liao S.C., Luo S.F. – Sequencing Analysis of cDNA Clones Encoding the American Cockroach Cr-PI Allergens. *J. Biol. Chem.*, 1996, 271, 17937-17943.
12. Wu C.H., Lee M.F., Wang N.M., Luo S.F. – Sequencing and Immunochemical Characterization of the American Cockroach Per a 3 (Cr-PI) Isoallergenic Variants. *Molecular Immunol.*, 1997, 34, 1-8.
13. Asturias J.A., Arilla M.C., Gomez-Bayon N., Martinez A., Martinez J., Palacios R. – Recombinant tropomyosin from American Cockroach. *Allergy*, 1998, 43, 29-30.
14. Vailes L.D., Kinter M.T., Arruda L.K., Chapman M.D. – High-level Expression of Cockroach Allergen Bla g 4, in *Pichia pastoris*. *J. Allergy Clin. Immunol.*, 1998, 101, 274-280.

