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Cross-reactivity between cockroach allergens and arthropod, nematode and mammalian allergens

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SUMMARY

Cockroaches (CRs) are major indoor allergens that can pose a serious risk for respiratory allergies in sensitized subjects. Recent evidence suggests that CR allergens cross-react with allergens present in insect and other invertebrates. IgE antibodies of insect-allergic subjects were shown to react with silverfish, CR and chironomid extracts. Cross-reactivity between house dust mites, silverfish, CR and chironomids and between cockroach and the reduviid, German CR and chironomids, and CRs crustacea species was established by RAST inhibition. There is some evidence that CR cross-reacts with the nematodes *Anisakis* and *Ascaris*. No cross-reactivity has been demonstrated between CR and mammalian allergens. The protein implicated in the cross-reactivity of CR with invertebrate allergens is tropomyosin. Tropomyosin has been identified as a major allergen in shrimp and other crustacea, CR, dust mites, and squid; these tropomyosins show substantial amino acid homology as well as immunological cross-reactivity. Clinical relevance of these cross-reactivities is suggested.

KEY-WORDS: Cockroaches. – Cross-reactivity. – Arthropodes. – Nematodes. – Tropomyosin.

RÉSUMÉ

Réactivité croisée entre les allergènes des blattes et les allergènes des arthropodes, des nématodes et des mammifères. – Les blattes sont un allergène domestique majeur qui constitue un risque sérieux d'allergie respiratoire chez les sujets sensibilisés. On a récemment montré l'existence d'une réactivité croisée entre les allergènes des blattes et ceux des insectes et d'autres invertébrés. Les anticorps IgE des sujets allergiques aux insectes réagissent aux extraits de lépisme (poisson d'argent), de blattes et de chironomides. Par inhibition du RAST, a été démontrée une réactivité croisée entre l'acarien de la poussière de maison, de lépisme, la blatte et les chironomides, et entre la blatte et les reduviides, la blatte germanique et les chironomides, et la blatte et l'espèce des crustacés. Il semble aussi qu'existe une réactivité croisée entre les blattes et les nématodes *Anisakis* et *Ascaris*. Aucune réaction croisée n'a été démontrée entre les blattes et les allergènes des mammifères. La protéine impliquée dans la réactivité croisée des blattes avec les allergènes d'invertébrés est la tropomyosine. La tropomyosine a été identifiée comme l'allergène majeur de la crevette et d'autre crustacés, des blattes, des acariens de la poussière et du calmar ; ces tropomyosines ont une forte homologie des amino-acides aussi bien qu'une réactivité croisée immunologique. Il est suggéré que ces réactivités croisées aient des implications cliniques.

MOTS-CLÉS: Blattes. – Réactivité croisée. – Arthropodes. – Nématodes. – Tropomyosine.

INTRODUCTION

As is evident from reports in the literature [1, 2] as well as presentations in this symposium, cockroaches (CRs) are major indoor allergens that can pose a serious risk for respiratory diseases such as allergic asthma in sensitized individuals. Indeed, in some inner-city areas in the United States CR is the single most important environmental factor with regard to the induction of asthma. CRs belong to the Phylum *Arthropoda*, class *Insecta*, order *Blattaria*, family *Blattidae* or *Blattellidae*. There are at least eight different CR species thought to be important clinically as

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causes of respiratory allergies [2]. A number of allergens have been identified, particularly in the German CR, *Blattella germanica* (Bla g 1, Bla g 2, Bla g 4, Bla g 5) and the American CR, *Periplaneta americana* (Per a 1, Per a 3, Per a 7) as these are species that have been most thoroughly investigated. Allergen cross-reactivity among American and German CR proteins has been established [1, 2], and probably occurs in most CR species investigated. However, not all allergens present in one particular species are expressed in other species. Recent evidence that CR allergens may cross-react with allergens present in insects and other invertebrates adds additional importance to CR in the induction of allergic reactions.

CROSS-REACTIVITY OF COCKROACHES WITH OTHER ARTHROPODS

The arthropods that have been most frequently investigated for cross-reactivity with CRs include members of classes *Insecta* and *Arachnida* such as caddis fly, may fly, moth and butterflies, non-biting midges (*Chironomidae*), the reduviid *Triatoma infestans* and housedust and storage mites [2, 3]. Attempts to define allergenic relationships between arthropod allergens, CRs in particular and mites have yielded contradictory results [2]. The problem with many of these studies is that cross-reactivity was neither directly determined nor allergens identified. Witteman *et al.* [4] demonstrated that IgE antibodies of insect allergic subjects react with silverfish, CR and or chironomid extracts. RAST inhibition confirmed cross-reactivity between housedust mites and silverfish, CR and chironomid extracts. Alonso *et al.* [3] demonstrated cross-reactivity between the CR (*Periplaneta americana*) and the reduviid (*Triatoma infestans*) by RAST and RAST inhibition. Additional studies by Pascual and coworkers using CAP-inhibition and immunoblot inhibition analysis demonstrated several common IgE binding components between German CR and chironomids [5]. Another member of the *Arthropoda phylum*, although less closely related to CRs than members of the *Insecta* and *Arachnida* class, is the crustacea family. The crustacea are composed of many edible sea creatures most notably shrimp, crab, lobster, and crawfish; these crustaceans are of particular interest since they are major food allergens. IgE binding components present in boiled Atlantic shrimp and German CR were shown to cross-react [6]; similar studies [7] demonstrated that CR antigens cross-react with several crustacea species. Although CR allergens were not investigated, a related study by Eriksson *et al.* [8] reported cross-reactivity between the larvae of chironomids (non-biting midges) and shrimp.

CROSS-REACTIVITY OF COCKROACHES WITH OTHER INVERTEBRATES

Pascual *et al.* [5] tested a serum pool of 18 pediatric patients with specific IgE to *Anisakis simplex* and sera from 21 German CR sensitive pediatric patients for IgE reactivity by immunoblot and immunoblot inhibition to determine whether the observed association of sensitization to nematodes and arthropods was due to immunological cross-reactivity or multiple sensitization. Immunoblot of *Anisakis* was partially inhibited with Chironomids and German CR extracts. A related study [9], investigated IgE antibody reactivity to a variety of arthropod insect inhalant allergens and the nematodes *A. simplex* and *Ascaris suis*. Anti-chicken tropomyosin demonstrated tropomyosin was the IgE binding component in arthropod and nematod extracts suggesting that this cross-reacting allergen was present in a variety of extracts from insects, mites, crustaceans, mollusks and parasites.

There is no evidence that CR and mammalian allergens cross-react even though Bla g 4 and important animal allergens such as murine urinary proteins, beta-lactoglobulin (milk), and dog, cow and horse allergens are members of the ligand-binding protein family known as lipocalins or calycins [1]. Although the sequence homology among these molecules is only about 20 %, their three-dimensional structure is conserved. However, no immunological cross-reactivity or common allergenic epitopes between CR and these mammalian allergens have been demonstrated.

NATURE OF THE CROSS-REACTING ALLERGEN: TROPOMYOSIN

Tropomyosin molecule is associated with the thin filament of muscle and microfilaments in many non-muscle cells and plays a regulatory role in muscle contraction. The function of tropomyosin in non-muscle cells is not well understood, but it is generally believed to participate in the regulation of cell morphology and motility. In muscle, two parallel alpha-helical tropomyosin molecules are wound around each other forming a coiled-coil structure. Coiled-coil proteins are characterized by hapted repeats with hydrophobic amino acids spaced every four and then three residues apart.

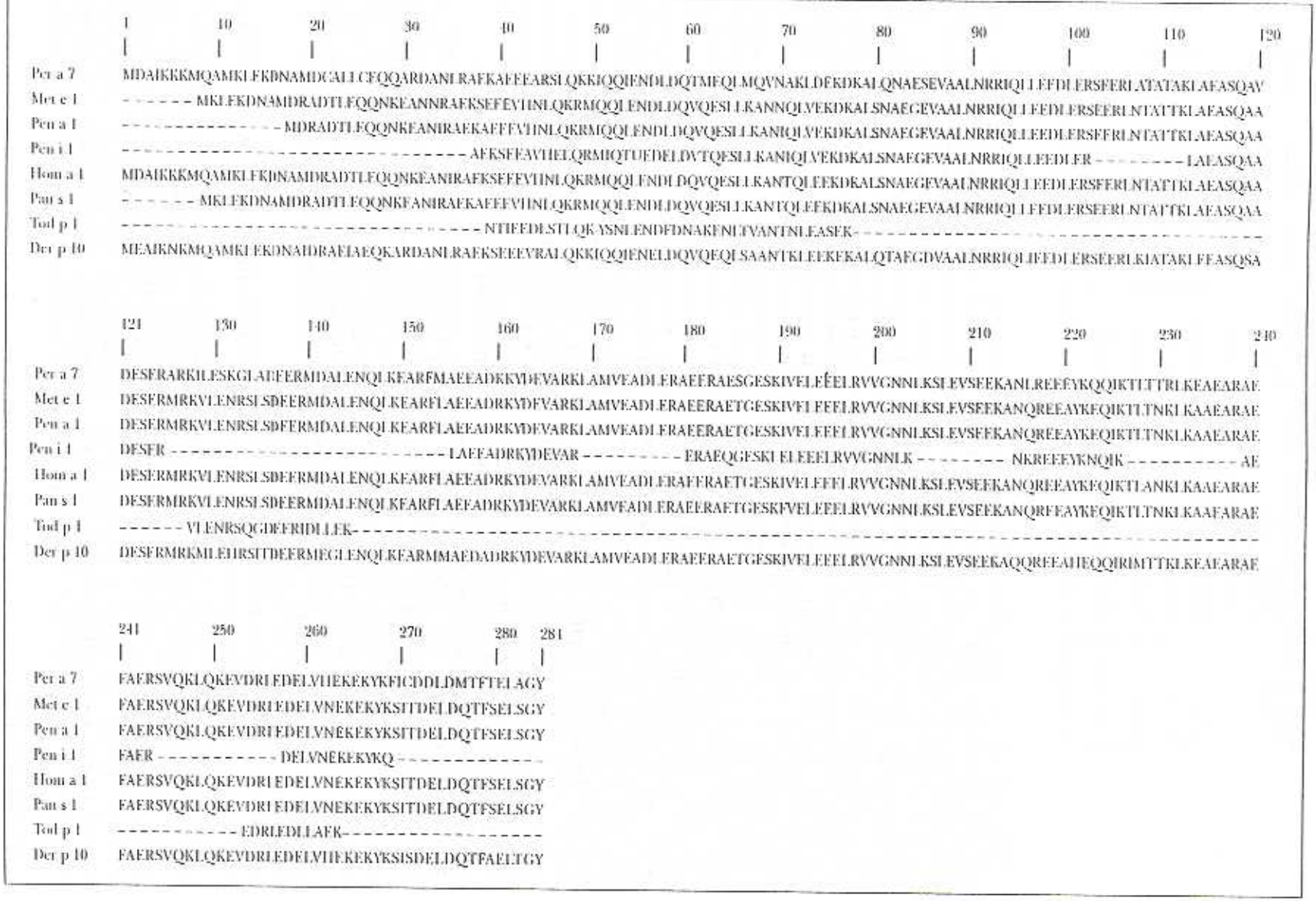

Fig. 1. – Amino acid sequences of allergenic Tropomyosins : American cockroach *Periplaneta americana* (Per a 7) [16], greasy-back shrimp *Metapenaeus ensis* (Met e 1) [12], brown shrimp *Penaeus aztecus* (Pen a 1) [14], Indian shrimp *P. indicus* Pen i 1 [11], spiny lobster *Panulirus stimpsoni* (Pan s 1), American lobster *Homarus americanus* (Hom a 1) [13], Pacific flying squid *Todarodes pacificus* (Tod p 1) and house-dust mite *Dermatophagoides pteronyssinus* (Der p 10) [17].

Table I. – Important tropomyosin allergens identified.

Source		*Allergen*	*Reference*
Shrimp	*Penaeus aztecus*	Pen a 1	Daul *et al.*, 1994
	Penaeus indicus	Pen i 1	Shanti *et al.*, 1993
	Metapenaeus ensis	Met e 1	Leung *et al.*, 1994
Mite	*Dermatophagoides farinae*	Der p 10	Aki *et al.*, 1995
Squid	*Todarodes pacificus*	Tod p 1	Miyazawa *et al.*, 1996
Spiny lobster	*Panulirus homarus*	Pan h 1	Leung *et al.*, 1998
Cockroach	*Periplaneta americana*	Per a 7	Asturias *et al.*, 1999 Santos *et al.*, 1999

Tropomyosin was first identified as a major allergen in shrimp based on the amino acid sequence homology of shrimp tropomyosin with tropomyosin from the fruit fly *(Drosophila melanogaster)* [10-12]. These findings are supported by demonstration of allergenic tropomyosins in other Crustacea species such as Florida lobster *(Panulirus argus)* and American lobster *(Homarus americanus)* [13]. Furthermore, IgE and Pen a 1-specific monoclonal antibodies detect Pen a 1-like allergens in crawfish *(Procambarus clarkii)*, crab *(Callinectes sapidus)* and Florida lobster [14]. In a cDNA library of brown shrimp *(Penaeus aztecus)* [14] the cDNA sequences of IgE-antibody positive clones could be aligned with various tropomyosins including that of greasy-back shrimp *(M. ensis)* tropomyosin (Met e 1), [12]. The deduced amino acid sequence is shown in figure 1. Recently, the analysis of IgE binding regions of Pen a 1 were performed. First, a recombinant library of randomly generated peptides, was screened with a serum pool of shrimp-allergic subjects. Four IgE-antibody positive clones were tested by grid immunoblotting using individual sera; three IgE binding areas. Pen a 1 (136-148), Pen a 1 (157-179), and Pen a 1 (262-282) were identified [14]. Second, 46 overlapping peptides (length: 15 amino acids, offset: 6 amino acids) synthesized on a cellulose membrane (SPOTs epitope mapping system, Genosys) were screened for IgE reactivities with sera of shrimp-allergic subjects and I^{125} labeled anti-IgE. Based on high frequency of detection and high magnitude of the IgE reactivity, five major IgE binding regions were identified.

Recently, tropomyosin from *P. americana* has been identified as a major CR allergen [15, 16]. Arruda *et al.* [15] screened a cDNA library from *P. americana* with IgE antibodies from a serum pool of 14 CR allergic asthmatic children. Five positive clones were isolated with the same sequence coding for protein with a high degree of homology to tropomyosin. The amino acid sequence deduced from the cDNA was 284 residues long and showed 80 %, 81% and 82 % identity to tropomyosin from the dust mites, *D. pteronyssinus, D. farinae*, and shrimp, *Metapenaeus ensis*, respectively. Using 53 individual sera from CR-allergic children, 27 (51.1 %) patients showed IgE binding to CR tropomyosin, demonstrating that tropomyosin is a major CR allergen. Concurrently, Asturias [16] *et al.* have cloned *P. americana* tropomyosin allergen and expressed it in *E. coli*. The sequence of this protein compared to other tropomyosins is shown in figure 1. The protein demonstrated substantial homology with other arthropod tropomyosins from shrimp and mite (80 % identity), but much less homology with vertebrate tropomyosin (50 % identity). ELISA and western blot inhibition experiments using natural and recombinant purified tropomyosins from shrimp, mite and CR demonstrated IgE antibody cross-reactivity to these tropomyosin allergens suggesting that this molecule is a common allergen among invertebrates.

Tropomyosin is also a major allergen in dust mite [17], squid [18], and other mollusks [19]. Indeed, cross-reactivity between CR and insects; CR, nematodes and crustacea; crustacea and insects has been attributed to the tropomyosin molecule [2, 9]. Tropomyosins identified as important invertebrate allergens are summarized in table I.

CLINICAL RELEVANCE AND CONCLUSIONS

Van Ree *et al.* investigated the sera of 17 patients receiving immunotherapy of house dust mite allergy for IgE antibodies against snail and shrimp [20]. While the average IgE response to mite Der p 1 and Der p 2 did not alter significantly, the average response to snail showed a significant increase even though many subjects had not been expo-

sed to snails. This included two sera conversions from negative to strongly positive. Three patients had a positive RAST for shrimp. In two of the three snails/shrimp positive sera, IgE antibodies against cross-reactive tropomyosin were demonstrated. The two patients also had a positive skin prick test reaction for shrimp and demonstrated oral allergy syndrome after eating shrimp. In another unpublished study by de Blay and co-workers, 6 patients became allergic to mite and German CR tropomyosin subsequent to their sensitization to crustacea (F. de Blay, personal communications). These studies suggest that the induction of IgE during mite immunotherapy may cause allergy to foods of invertebrate animal origin or alternatively sensitization to crustacea may result in development of allergy to mites and CRs.

In conclusion, it is well accepted that CR is one of the most important indoor allergens, particularly in North America [1, 2]. There is accumulating evidence that CR allergens cross-react with other insect and mite aeroallergens, and other members of the *Arthropod* group particularly shrimp and other crustacea, as well as with nematodes. The molecule that accounts for much of this cross-reactivity is the muscle protein, tropomyosin. This molecule has a highly conserved coiled-coil structure stabilized by hydrophobic interactions that is important in muscle contraction. Because tropomyosin appears to be an allergen in so many invertebrate species, one might speculate that it is a pan-allergen of invertebrates. Such a molecule could be very useful for understanding the structure to functional relationship of allergenic activity. Since tropomyosin appears to be such a potent and widespread allergen, identification of structural features that contribute to its allergenicity will help advance our understanding of the relationship of protein structure to allergen function.

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