

Investigating cockroach allergens: Aiming to improve diagnosis and treatment of cockroach allergic patients



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ABSTRACT

Cockroach allergy is an important health problem associated with the development of asthma, as a consequence of chronic exposure to low levels of allergens in susceptible individuals. In the last 20 years, progress in understanding the disease has been possible, thanks to the identification and molecular cloning of cockroach allergens and their expression as recombinant proteins. Assays for assessment of environmental allergen exposure have been developed and used to measure Bla g 1 and Bla g 2, as markers of cockroach exposure. IgE antibodies to cockroach extracts and to specific purified allergens have been measured to assess sensitization and analyze association with exposure and disease. With the development of the field of structural biology and the expression of recombinant cockroach allergens, insights into allergen structure, function, epitope mapping and allergen–antibody interactions have provided further understanding of mechanisms of cockroach allergic disease at the molecular level. This information will contribute to develop new approaches to allergen avoidance and to improve diagnosis and therapy of cockroach allergy.

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1. Origins of cockroach allergens

Approximately 4000 species of cockroaches are distributed worldwide, with most species being inhabitants of tropical forests around the world. Well adapted to a wide and diverse range of habitats, cockroaches have been very successful in surviving throughout evolution [1]. A recent study showed that the plasticity of their sensory system, reflected in changes in taste neurons, allows cockroaches to develop glucose-aversion as a novel behavior that offers protection against toxic baits containing insecticide and glucose added as a phagostimulant [2]. Cockroaches are source of proteins that contribute to asthma and other allergic diseases [3,4]. The description of cockroach allergens and their association with human diseases will be the focus of the present review.

Despite the abundance of cockroach species, only few have adapted to human habitats. Domiciliary cockroaches include approximately 25 species from the families Blattellidae, Blattidae and Blaberidae (Order Blattaria, Class Insecta and Phylum Arthropoda) [5]. Two species, German cockroach (*Blattella germanica*) and American cockroach (*Periplaneta americana*) predominate in temperate and tropical areas, respectively, and have been the focus of allergy research. German cockroach is commonly found in cool and dry climates (i.e. Europe and USA), whereas the

larger American cockroach (35–53 mm long versus 16 mm) prefers hot and humid conditions (i.e. Brazil, Taiwan and Thailand) [6].

The World Health Organization and International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature database (www.allergen.org) reports only allergens from German and American cockroaches, which have been approved by the sub-committee for systematic allergen nomenclature. However, other cockroach species have been found in homes from different parts of the world, and potential for cross-reactivity among homologous allergens from different species is expected. In Thailand, the predominant cockroach species is the American cockroach [6]. A study of sixty households of cockroach allergic patients in Bangkok identified *P. americana* as the most common (72%) followed by *Supella longipalpa* (brown-banded, 2.75%), *Periplaneta brunnea* (brown, 0.78%), *Periplaneta australasiae* (Australian, 0.78%), *Neostylopyga rhombifolia* (harlequin, 0.78%) and *B. germanica* (0.39%) [7]. In homes from Tokyo areas, *Periplaneta fuliginosa* (dusky or smoky brown) is the predominant species [8]. Four cockroach species infested homes in Seoul (Korea): the German (36.2%), American (22.4%), Japanese (*Periplaneta japonica*) (1.1%), and dusky brown (1.7%) cockroaches [9].

Geographical distribution of domiciliary cockroach species will determine the specificity and cross-reactivity of the IgE antibody responses worldwide. In accordance with this, a study reported shared allergenic activity in Asian (*Blattella asahinai*), German, American and Oriental (*Blatta orientalis*) cockroach species using

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sera from German cockroach sensitive individuals [10]. Cross-reactivity among *P. americana*, *P. fuliginosa* and *B. germanica*, specifically to tropomyosin, has also been reported [11].

2. Allergen exposure, sensitization and clinical disease

Positive skin tests and specific IgE to cockroach have been reported in patients with allergic rhinitis and atopic dermatitis, and eczematous skin reactions to cockroach extract have been described in patients with atopic dermatitis [12,13]. However, the most consistent association of cockroach allergy with human diseases has been found with asthma.

Cockroach allergy has been recognized to contribute to the development of asthma for almost 50 years. In 1964, Bernton and Brown were the first to report positive skin test responses to cockroach allergen (44%) in a study involving 755 allergy clinic patients living in New York [14]. Subsequent work by Kang et al. confirmed the causal relationship between cockroach allergy and asthma by showing early, late-phase, and dual bronchoconstriction after inhalation of cockroach extract by sensitized asthmatic patients [15].

One major step towards a better understanding of cockroach allergy was the development of methods for measuring cockroach allergens in environmental samples, making it possible to investigate the relationship of exposure to sensitization and development of clinical disease. This has been possible thanks to monoclonal antibodies (mAb) initially developed against extracts or purified natural, and more recently against recombinant, allergens. Monoclonal (mAb)-based ELISA assays for quantitation of Bla g 1 and Bla g 2 have been used worldwide, and these allergens have proven to be excellent markers of cockroach allergen exposure in environmental and avoidance studies [16]. More recently, multiplex array technology has been applied for simultaneous measurement of multiple allergens in dust samples, reducing considerably the time and volume of sample needed to assay a group of allergens [17].

Studies on the biology of cockroach allergens demonstrate that Bla g 1 and Bla g 2, together with Bla g 4, are allergens secreted or excreted by cockroaches. Other allergens with structural functions, like the tropomyosins from Group 7 or allergens from Groups 6 and 8, result from degradation of dead bodies [3]. These dried secretions and remains of body parts could be the form in which cockroach allergens become airborne and are inhaled, causing sensitization in genetically predisposed individuals. The accumulation and persistence of stable cockroach allergens in the home could result in low levels of chronic exposure that is associated with asthma.

Exposure to cockroach has been associated with wheezing and asthma morbidity, especially in subjects who are sensitized. The National Cooperative Inner City Asthma Study (NCICAS) provided very relevant information on the role of cockroach allergy in asthma. The initial study by Rosenstreich et al. revealed that inner-city children with asthma who were both sensitized to cockroach and exposed to high levels of cockroach allergen in their homes presented higher asthma morbidity, as indicated by more hospitalizations, more medical visits, and more reported symptoms [18]. Several studies in more recent years have confirmed the association of cockroach exposure and increased asthma morbidity in inner-city areas in the United States and also in other countries [19–22]. Exposure to low levels of Bla g 1 and Bla g 2 allergens has been associated with wheezing among infants in the first 3 months of life and with increased proliferative T cell responses [23]. Bla g 2 was able to induce IgE production at levels of exposure 10–100 fold lower (<1 µg/g dust) than cat and mite allergens [24]. Threshold levels of cockroach exposure above which susceptible individuals would be at increased risk for sensitization or asthma symptoms have been defined as 2 and 8 U/g of dust of allergen, respectively,

for both Bla g 1 and Bla g 2 [1,18,25]. Other reports have shown association of cockroach allergen exposure with persistent childhood wheezing [26] and severe asthma [27].

In inner-city communities in the United States, Wang et al. demonstrated that having specific IgE to cockroaches, and also to dust mites, was associated with an increased risk for asthma hospitalizations and corticosteroid use, in a population of asthmatic children [28]. Results of a recent inner-city birth cohort study showed that prenatal exposure to cockroach allergen was associated with a greater risk of sensitization by the age of 5–7 years. This risk was increased by exposure to airborne nonvolatile combustion byproducts (polycyclic aromatic hydrocarbons), with children null for the Glutathione-S-transferase m 1 (GSTM1) mutation particularly vulnerable [29]. Many studies highlight the importance of cockroach allergy in inner-city environments. However, cockroach-induced asthma occurs whenever substandard housing permits cockroach infestation. This includes rural and semirural areas, suburbs, and small towns and cities across the world [30]. In conjunction, these studies point to an important role of exposure and sensitization to cockroach in inducing earlier and more severe symptoms of asthma, at a much lower dose of allergen exposure as compared to other allergens sources.

Specific properties of cockroach allergens, including proteolytic activity, have been proposed to contribute to more severe disease associated with cockroach exposure and sensitization. There is evidence that the biological function of the allergen has direct effects on the lung. Effects of proteases from cockroach extracts on promoting disease have been observed for a long time. An increase in bronchial airway epithelial permeability by cockroach extracts was reported, similarly to the effect observed for dust mite proteases [31]. Subsequently, a serine protease activity with a direct pro-inflammatory effect on airway epithelial cells was proven using specific inhibitors [32]. The mechanism by which cockroach proteases increased IL-8 expression in human bronchial epithelial cells involved activation of protease-activated receptor (PAR)-2 and extracellular-signal-regulated kinase [33]. Further studies are needed to better understand the effect of cockroach proteases in allergic disease. Interestingly, despite the role of proteases in cockroach allergy [34–37], most cockroach allergens identified by molecular cloning to date are non-proteolytic, including the unusually inactive aspartic protease Bla g 2 [38,39]. The only exception is allergen Per a 10, which has serine protease activity [40].

Efforts have been made to reduce environmental allergen exposure in homes of patients with cockroach-induced asthma, which ultimately could lead to improvement of symptoms. Cockroach allergens may persist in the environment up to several months following eradication of the insects [41]. Researchers from the Inner City Asthma Studies (ICAS) were the first to demonstrate that environmental interventions could effectively reduce asthma symptoms [42]. One year of a comprehensive controlled intervention strategy including professional cleaning, bait traps, insecticides, and HEPA filters, could decrease cockroach allergen levels. This change significantly correlated with decreased wheeze and less nighttime asthma symptoms, and fewer missed schooldays [42]. These clinical improvements lasted for 1 year after the monitored intervention had ended. The study demonstrates that continuous efforts might be necessary for sustained cockroach allergen reduction. Therefore, this strategy may not be feasible in real life conditions.

A recent study investigated the effectiveness of adding Omalizumab, a monoclonal anti-human IgE antibody, to guideline-directed care for inner-city children, adolescents, and young adults with allergic asthma [43]. Omalizumab is indicated for patients who have moderate-to-severe allergic asthma that is not controlled with implementation of the higher treatment steps of

the most recent guidelines from the National Asthma Education and Prevention Program (NAEPP) (Expert Panel Report 3). Treatment with Omalizumab resulted in a significant decrease in asthma symptoms, reduction in number of asthma exacerbations, fewer hospitalizations, and reduced need for inhaled glucocorticoids to maintain asthma control, as compared to placebo. In addition, use of Omalizumab caused marked reduction in seasonal asthma exacerbations, both in spring and fall. A striking finding of this study was that the subgroup of patients who were both sensitized and exposed to cockroach (Bla g 1 levels ≥ 2 U/g) in their homes benefited most with Omalizumab treatment, with a 71.2% reduction in exacerbations, greater improvement of symptoms, and greater reduction of dose of corticosteroids, as compared to other participants. The authors suggest that the combination of sensitization and exposure to cockroach could serve as a criterion for indicating Omalizumab use for optimal effectiveness and cost benefits [43].

Sensitization to individual cockroach allergens has been evaluated, aiming at identifying markers for severity of disease among cockroach allergic patients. Recombinant *P. americana* allergens (Per a 1 through 7, and Per 9), expressed in *Escherichia coli*, were used to investigate whether sensitization to different cockroach allergens would correlate with clinical manifestations and severity of disease among patients from Taiwan [44]. As expected, patients with more severe disease, presenting with persistent asthma and rhinitis, had higher serum levels of inflammatory cytokines interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), chemokine ligand 20 (CCL-20), and granulocyte macrophage colony-stimulating factor (GM-CSF), as compared with patients with allergic rhinitis only, and non-allergic subjects. Interestingly, IgE-binding to Per a 2 was more frequent among the group of patients with persistent asthma, as compared to patients with rhinitis only (81% versus 45%, $p < .05$). In contrast, 80% of rhinitis patients had IgE-binding activity to Per a 9, as compared with only 28.5% among the patients with asthma ($p < .01$). The results suggested that sensitization to Per a 2 could be a marker of more severe airway disease [44].

3. Recombinant cockroach allergens: from molecular cloning to a better understanding of cockroach allergy

Significant progress in our understanding of cockroach allergy was initiated by the identification, molecular cloning and measurement of allergens associated with development of the disease [1,3]. The subsequent analysis of the immunological responses to these allergens has contributed over the years to gain further insight into cockroach allergy. This breakthrough was possible thanks to the development of sophisticated technologies for molecular cloning, expression of recombinant proteins and analysis of the structural biology of cockroach allergens (Table 1). In the 1990s, cDNA libraries were made by reverse transcription of RNA isolated from cockroaches. Typically, these libraries would be screened with radiolabelled nucleotide probes designed from partial sequences of purified proteins. Alternatively, cDNA would be used to construct expression libraries, suitable for identifying completely unknown proteins for which a specific antibody was available and used for screening. This later strategy was very successful for the molecular cloning of most of the known cockroach allergens, since IgE antibodies from cockroach allergic patients and allergen-specific monoclonal antibodies were available [1,3]. Some expression libraries were constructed by introducing cDNA into expression vectors, each consisting of a plasmid with a polylinker for cloning, an origin of replication and an antibiotic resistance gene. These vectors were transformed into bacteria for protein expression. Phagemid expression libraries were also used for the cloning of

Table 1
Methods associated with studies of cockroach allergens.

Identification
Radioimmuno-electrophoresis
Immunoblotting
Proteomic techniques
Measurement
ELISA
Polyclonal antibody production by immunization with allergen
Monoclonal antibody production by hybridoma technology
Multiplex array
Molecular cloning
Cloning of cDNA by PCR amplification
Screening of cDNA expression libraries
Expression
Prokaryotic expression system (<i>Escherichia coli</i>)
Eukaryotic expression system (<i>Pichia pastoris</i>)
Purification
Affinity chromatography
Size exclusion chromatography
Ion-exchange chromatography
Isoelectric precipitation
Ammonium sulfate fractionation
SDS-PAGE electrophoresis
Biological function
Primary structure: sequence homology analysis
Tertiary structure determination: X-ray crystallography
Functional assays (i.e. enzymatic activity)
Immunohistochemistry
RNA interference-mediated knock-down
Immunological effects
Mediator release assays
Cytokine release assays
T cell reactivity
PAR receptor activation
Epitope mapping
Linear epitopes: Recognition of allergen peptides or fragments
Conformational epitopes: Structure of allergen-antibody complexes
Site-directed mutagenesis
Production of hypoallergens
Site-directed mutagenesis
Antibody binding assays
CD spectra/X-ray crystallography
Mass spectrometry
Assessment of prevalence and IgE antibody titers
ELISA
Immunoblotting
Plaque immunoassay
Streptavidin ImmunoCAP assay
Diagnosis
Skin prick test with cockroach extracts
CAP Immunoassay
Immunotherapy
Administration of increasing doses of cockroach extracts

cockroach allergens. In this case, a phage carrying cDNA was used to infect bacteria, and plaques resulting from phage infection, instead of bacterial colonies, were screened using allergen-specific antibodies, mostly from cockroach allergic patients' sera, instead of nucleotide probes. The identified DNA was then used for *in vitro* allergen expression in systems such as *E. coli* or *Pichia pastoris*.

The availability of purified cockroach allergens, either from the natural source or recombinant, made possible the measurement of exposure and sensitization to specific allergens, and the assessment of their association with disease. Additionally, the analysis of the structural biology of cockroach allergens has provided further insight into mechanisms of disease.

4. Cockroach allergens

The first cockroach allergen to be cloned was Bla g 2 in 1995 [45]. Since then, the identification of German and American cockroach allergens has continued, and there are presently 10 Groups reported in the WHO/IUIS Allergen Nomenclature database. The allergens are Bla g 1, Bla g 2, Bla g 3, Bla g 4, Bla g 5, Bla g 6, Bla g 7 and Bla g 8 from *B. germanica*, and Per a 1, Per a 3, Per a 6, Per a 7, Per a 9 and Per a 10 from *P. americana* (www.allergen.org) (Table 2). Although only Groups 1 and 7 have been reported to be cross-reactive, additional cross-reactivity among allergens from other Groups, such as Group 6, cannot be discarded. In addition to the cockroach allergens officially reported in the WHO/IUIS database, other related proteins have been identified. For example, some *P. americana* proteins share homology with allergens from the *B. germanica* Groups 2 and 4 (GenBank accession numbers AY792947 and AY792948), and with the mite allergens from Group 2, 3 (trypsin) and 13 (fatty acid binding protein) (GenBank accession numbers AY792953, AY792954 and AY792955). The Bla g 2 homolog from *P. americana* (44% shared identity) was identified by Dr. FT Chew [38]. A Bla g 4 homolog from *P. americana*, with a typical lipocalin fold, has also been reported [46]. Another study described a troponin-T from American cockroach with 17% prevalence of IgE reactivity, but it is not yet present in the official database [47].

High degree of sequence polymorphisms has been described for Bla g 1, Bla g 2, Bla g 4, Bla g 5 and Bla g 7 by PCR amplification of

cDNA encoding for cockroach allergens [48,49]. However, caution is needed before accepting these variants identified by PCR as naturally occurring allergens, since the technique could introduce errors occurring during DNA amplification. The WHO/IUIS Allergen Nomenclature Sub-Committee reviews submissions of new allergens and requests proof of IgE antibody recognition of new variants or isoallergens before their acceptance to the database.

Finally, the existence of other relatively high-molecular weight (MW) cockroach allergens, not yet cloned or identified, has been known since 1995 [50]. Recently, using a combination of proteomic techniques and bioinformatic allergen database analysis, a total of ten new IgE-binding proteins were identified in whole-body extract of the German cockroach in a Taiwanese population. These include the high molecular weight protein vitellogenin (which breaks down to fragments of up to 97 kDa) [51]. Proteins were separated by 2-DE, and IgE-binding proteins were analyzed by nanoLC-MS/MS or N-terminal sequencing analysis. Among these new proteins, aldolase, arginine kinase, enolase, Hsp70, triosephosphate isomerase and vitellogenin have been reported as allergens in species other than *B. germanica*, with potential for cross-reactivity [51]. Another proteomics study recently identified twelve new IgE-reactive components from German cockroach fecal extract in a Korean population. Most of these allergens were found to be digestive enzymes such as α -amylase, trypsin, chymotrypsin, metalloprotease and midgut carboxy peptidase A. Alpha-amylase was found to be important, with a 41.4% (12/29) prevalence of specific IgE in serum from German cockroach-sensitized subjects

Table 2
Systematic nomenclature of cockroach allergens.

Allergen	M.W. ^a	Function/homology	IgE prevalence (%) ^e	GenBank accession #	PDB ID	Reference(s)
<i>Blattella germanica</i>						
Bla g 1	~25–90	Midgut microvilli	20–40			[45,54,72,73]
Bla g 1.0101	46, 21	Protein homolog		AF072219, AF072221		[73]
Bla g 1.0102	25–37			L47595		[72]
Bla g 1.0201	56			AF072220		[73]
Bla g 2	36	Inactive aspartic protease	40–70	U28863	1YC9, 2NRS ^b , 3LIZ ^b	[38,39,45,86–88]
Bla g 3	79	Hemocyanin	n.p.	GU086323		n.p.
Bla g 4	21	Lipocalin	17–40	U40767	3EBK ^c	[46,57]
Bla g 5	23	Glutathione S-Transferase	35–68	U92412		[63]
Bla g 6	17	Troponin C	14			[66]
Bla g 6.0101				DQ279092		[66]
Bla g 6.0201				DQ279093		[66]
Bla g 6.0301				DQ279094		[66]
Bla g 7	33	Tropomyosin	18	AF260897		[70]
Bla g 8		Myosin light chain	14	DQ389157		[66]
<i>Periplaneta americana</i>						
Per a 1	~25–45	Midgut microvilli	5–9, 54, 100			[12,58,59,74–76]
Per a 1.0101	26	Protein homolog		AF072222		[75]
Per a 1.0102	26			U78970		[74]
Per a 1.0103	45			U69957		[76]
Per a 1.0104	31			U69261		[74]
Per a 1.0201	51			U69260		[74]
Per a 3	46–79	Arylphorin/hemocyanin	26–95			[61,62]
Per a 3.0101	79			L40818		[61]
Per a 3.0201	75			L40820		[61]
Per a 3.0202	56			L40819		[62]
Per a 3.0203	46			L40821		[62]
Per a 6	17	Troponin C	n.p.	AY792950		[66]
Per a 7	33	Tropomyosin	13–54			[12,53,54,69]
Per a 7.0101				Y14854		[69]
Per a 7.0102				AF106961		[53]
Per a 9	43	Arginine kinase	80–100	AY563004 ^d		[64]
Per a 10	28	Serine protease	82			[40]

n.p. Not published.

^a Molecular weight calculated from sequence.

^b X-ray crystal structures of Bla g 2 in complex with monoclonal antibodies that inhibit IgE antibody binding.

^c The structure of a *P. americana* homolog has been reported under the Protein Data Bank Accession number 3EBW.

^d Accession # not yet published in the WHO/IUIS Allergen Nomenclature Database.

^e Prevalence of IgE reactivity varies depending on the population and the geographical area (see Section 5 for specific details).

[52]. The finding of this additional relatively high prevalent allergen is interesting given the lack of immunodominance of known cockroach allergens.

5. Prevalence of sensitization to cockroach allergens

Cockroach allergens, purified from natural sources or expressed as recombinant proteins, became tools for assessment of prevalence of sensitization and measurement of allergen-specific IgE antibody titers in allergic populations. The original methods, radioallergosorbent tests (RAST) and radioimmunoassays (RIA), were substituted with time by ELISA, ImmunoCAP and multiplex array assays [53–56]. Recombinant cockroach allergens have been very useful for *in vitro* studies, which revealed that patients present variable allergen sensitization profiles; no single major allergen appears to account for most of the IgE reactivity to cockroach (Table 2). Also, it has become apparent that the importance of individual allergens in causing sensitization varies in different areas of the world, possibly under influence of sensitization to cross-reactive antigens.

In most studies carried out in the US, Bla g 2, Bla g 4 and Bla g 5 were reported to be the most important allergens, as judged by higher prevalence of sensitization to these allergens among cockroach allergic patients with asthma [45,54,57,58]. Using streptavidin CAP and a multiplex flow cytometric assay, Satinover et al. demonstrated that a panel of 5 purified recombinant allergens (rBla g 1, rBla g 2, rBla g 4, rBla g 5, and rPer a 7) could identify 64% of cockroach allergic US patients, defined by a positive ImmunoCAP to *B. germanica* extract [54]. Prevalence of IgE antibodies was highest for rBla g 2 (54.4%) and rBla g 5 (37.4%) among the 118 sera analyzed. However, patterns of IgE antibody binding were unique to each subject, irrespective of their geographic location within the US. Prevalence of IgE antibodies to the other three allergens tested was lower: rBla g 1 (26%), rBla g 4 (17%), and rPer a 7 (13%) [54]. These results were in contrast with sensitization to indoor allergens from cat and mite, which shows a clear immunodominance for few allergens (Fel d 1 for cat and Groups 1 and 2 for mite). Further analysis revealed that the prevalence of IgE antibodies to rBla g 2 and rBla g 5 was 71% and 58%, respectively, among sera with high IgE levels to CR extract (3.5–100 IU/ml). These results agree with the prevalences of sensitization reported in the original molecular cloning studies [45]: 30% and 57% of 106 cockroach allergic patients had detectable IgE antibodies to natural Bla g 1 and Bla g 2, respectively. The prevalence was lower (19% and 42%, respectively) among patients with low RAST (<200 RAST units/ml; *n* = 48), and reached up to 40% and 70%, respectively, among patients with high RAST (>200 RAST units/ml; *n* = 58) [45]. In addition, positive intradermal skin tests at Bla g 2 concentrations as low as $10^{-3.5}$ µg/ml were observed [45]. In these earlier studies, recombinant Bla g 4 was recognized by 41% of cockroach allergic patients with high RAST, and by 31% of patients with a RAST value of 40–200 units/ml (*n* = 73 patients) [57]. These results indicate that, within the same population, prevalences of sensitization vary depending on the patients titers of specific IgE antibodies against cockroach extracts. Therefore, the classification of an allergen as major, according to a prevalence of sensitization larger than 50%, will depend on the population selected.

The prevalence of sensitization may also vary widely between populations, depending on the world location. IgE reactivity to Per a 1 ranges from 9% in Brazil and 54% in Taiwan, to 100% in Thailand [12,58,59].

Per a 3 was identified as a group of proteins with a wide range of skin test reactivity (26–95%). The relevance of this allergen in cockroach disease needs to be further evaluated [60–62].

Natural and recombinant Bla g 5 were recognized by 68% and 73% of 40 patients, respectively [63]. Biological reactivity of natural

Bla g 1 and Bla g 2, and rBla g 4 and rBla g 5 was proven by intradermal skin testing, showing positive immediate skin reactions (>8 × 8 mm wheal) down to 10^{-3} – 10^{-5} µg/ml of these purified allergens. Interestingly, sensitization in 95% of *B. germanica* allergic patients was demonstrated by measuring IgE binding to Bla g 1, Bla g 2, Bla g 4 and Bla g 5 in these selected cockroach allergic patients [63].

Groups 9 and 10 have emerged in the last years as potentially important cockroach allergens. An arginine kinase from *P. americana* was identified as a major allergen among cockroach allergic Thai patients and was assigned to Group 9 by the WHO/IUIS Subcommittee of Allergen Nomenclature [64]. All the patients (*n* = 25) had IgE recognizing the purified 40 kDa allergen by western blot. The arginine kinase from *P. americana* was cloned by Dr. FT Chew (GenBank accession number AY563004) and a *B. germanica*-homolog was also reported (GenBank accession number ABC86902). Group 10 comprises serine proteases, only reported as allergen for *P. americana*, with a 82% prevalence of sensitization among 45 cockroach allergic patients by intradermal skin testing with the purified protein in studies from India [40,65].

Finally, the remaining cockroach allergens are minor and belong to Groups 6, 7 and 8. These allergens are proteins which show homology to troponin C, tropomyosin and myosin light chain, respectively [66,67]. Three Bla g 6 isoallergens and Bla g 8 were cloned from a *B. germanica* cDNA expression library, were expressed as recombinant proteins in *Pichia pastoris*, and showed a 14% prevalence of IgE antibodies [66]. The Group 7 comprises homologues to tropomyosin, a pan-allergen among invertebrates, involved in muscle contraction [53,67–70]. Tropomyosin is considered a minor allergen among cockroach allergic patients from the US and Korea. A 13% prevalence was reported for 118 US cockroach allergic patients by streptavidin CAP assay [54], and only 18% (6/35) of sera from cockroach allergic patients in Korea showed IgE reactivity to natural Bla g 7 by ELISA [70]. Interestingly, IgE reactivity to cockroach tropomyosin has been shown to be much higher in certain areas of the world including Brazil and Africa, as it will be discussed later in this review.

6. Groups of cockroach allergens

6.1. The Group 1 of cross-reactive cockroach allergens

The Group 1 cockroach allergens comprises cross-reactive allergens from *B. germanica* and *P. americana* that were first purified by conventional biochemical techniques or using monoclonal antibodies raised against cockroach extracts [16,71,72]. These early studies showed that the native allergen had a different molecular weight (6–37 kDa) depending on the method used for purification [71]. In the late 1990s, the molecular cloning of Bla g 1 and Per a 1 revealed that both allergens, sharing a 52–72% amino acid sequence identity, had a novel primary structure constituted of multiple tandem repeats of ~100 amino acid residues [72–76]. Multiple clones containing different number of repeats were identified. The original clone Bla g Bd90K was the largest, with 14 tandem repeats, and was officially re-named as Bla g 1.0102 [72]. Dot-plot sequence analysis revealed that two consecutive amino acid repeats were encoded by one nucleotide repeat, and shared lower identity (26–29%) than alternate repeats (96–98%). These observations led to the hypothesis that Bla g 1 originated by duplication of a gene encoding for ~100 amino acids. The duplex would have diverged with time, and subsequently replicated several fold. In addition, N-terminal sequencing of the natural allergen showed cleavage by trypsin-like enzymes after arginine residues, resulting in a mixture of molecules of different size, containing a different number of amino acid repeats, all recognized by IgE [73,77]. These unique properties of the Group 1 allergens explained the wide

range of molecular weights observed upon purification of the natural allergen.

Bla g 1 is secreted in the midgut of the cockroach digestive system, especially by females and after a food intake [78,79]. A function of Bla g 1 in digestion and nutrient absorption was confirmed by a study performing RNA interference-mediated knock-down of this allergen in German cockroach [80]. In agreement with a digestive function, the Group 1 cockroach allergens shows homology to microvillar membrane associated proteins from the coleopteran *Tenebrio molitor* (GenBank accession number AY327800) and the mosquito *Aedes aegypti* (AEG12) (AY038041, AY050565) [81–83]. They also share homology with ANG12, a protein from *Anopheles gambiae* which, as AEG12, is induced after a blood meal in the midgut of adult female mosquitoes (accession number Z22925) [84]. Additional homologous G12 proteins from other insect species are present in the GenBank. The presence of Bla g 1 in fecal particles makes this molecule, together with Bla g 2, a good marker of cockroach allergen exposure.

6.2. The atypical aspartic protease Bla g 2

Bla g 2 is one of the most important cockroach allergens that elicits IgE antibody responses in 40–70% of cockroach allergic patients at low levels of exposure [24,45]. Bla g 2 shares homology with the proteolytic enzymes aspartic proteases (pepsin, renin, chymosin), is secreted in the digestive system (mainly in esophagus, proventriculus and gut), and excreted in fecal particles together with Bla g 1. Although it was originally assumed that Bla g 2 proteolytic activity could contribute to allergenicity, structural studies revealed that Bla g 2 is an inactive aspartic protease [38,39]. Typical aspartic proteases have a catalytic site at the bottom of a cleft between two almost symmetrical lobes. Although Bla g 2 has the same overall fold as aspartic proteases, it is not active, because the residues equivalent to the ones forming the conventional catalytic triads (D-T/S-G) are different in Bla g 2 (DST and DTS). Additional structural features impair enzymatic activity: (1) a larger distance between the catalytic aspartates, and (2) the presence of an extra phenylalanine (F75a) that blocks the access of substrates to the catalytic site, contributing to this auto-inhibitory mechanism of catalysis [38,39]. Regarding these structural features, Bla g 2 strongly resembles a group of inactive aspartic proteases called pregnancy associated glycoproteins (PAG), secreted in the chorion of female ruminants during pregnancy [85]. Similar amino acid substitutions to Bla g 2 have been observed in PAGs [86]. This finding was relevant at a time when a big emphasis had been placed on the importance of proteolytic activity on allergenicity, as observed for several allergens originally described (mite Groups 1, 3, 6 and 9). In contrast, Bla g 2 is a potent allergen without a conventional proteolytic activity. Multiple allergens are known not to be proteolytic enzymes, as it will be shown in the following sections for most of the known cockroach allergens.

Bla g 2 has two more structural features that may contribute to its allergenicity by conferring stability to the molecule. First, the presence of 5 di-sulfide bonds in contrast to only 2–3 in typical aspartic proteases. Second, the binding of an atom of zinc that coordinates the amino acids His155, His161, Asp303 (the only one conserved among aspartic proteases) and Asp307. These four residues are also present in the amino acid sequence of the *P. americana* and *Leucophaea maderae* Bla g 2-homologs. Both structural features may provide stability to Bla g 2 and influence its persistence in the environment. Chronic exposure to low Bla g 2 doses (1–10 µg/year) may explain why sensitization and exposure to Bla g 2 are associated with the development of asthma [1,24].

Recently, the antigenic structure of Bla g 2 was analyzed by X-ray crystallography and site-directed mutagenesis. First, epitopes overlapping with IgE antibody binding sites were identified by

solving the crystal structure of complexes of the allergen with fragments (Fab or Fab') of monoclonal antibodies that inhibit IgE antibody binding [87,88] (Fig. 1). Second, mutagenesis of residues in the epitopes and antibody binding analysis allowed to identify amino acids involved in antigen–antibody recognition [90]. These studies led to the expression of hypoallergens as potential tool for immunotherapy, and revealed interesting mechanisms of allergen–antibody interaction.

6.3. Group 3: hemocyanin homologs

Per a 3 is an hexameric protein that shares homology with arylphorins or insect storage proteins (20–34%), insect juvenile hormone-suppressible proteins (31–36%) and arthropod hemocyanins (30–35%) [60,61,91] (Table 2). Per a 3 was originally identified as two antigenic components of 72 and 78 kDa in a partially purified fraction from cockroach extracts (Cr-PI). Recently, a 79 kDa homolog, Bla g 3, has also been reported in the WHO/IUIS official list of allergens. Per a 3 was described as a major American cockroach allergen because the Cr-PI fraction induced 73% (11/15) and 82.6% (19/23) skin test reactivity in cockroach allergic patients in two studies by the same group [60,61]. However, several Per a 3 clones were isolated from a λgt22A cDNA library with a wide range of skin test reactivities (26–95%) [61,62]. Therefore, the relevance of this allergen remains controversial, since prevalence largely differs among different variants or isoallergens.

6.4. The insect lipocalin Bla g 4

Cockroach allergens from Group 4 are insect lipocalins. In contrast, most lipocalin allergens are of mammalian origin, and include: Bos d 2 and Bos d 5 from cow; Can f 1, Can f 2, Can f 4 and Can f 6 from dog; Equ c 1 and Equ c 2 from horse; Fel d 4 and Fel d 7 from cat, Mus m 1 from mouse and Rat n 1 from rat [92–97]. The major dog allergen Can f 6, with 57% amino acid identity to Equ c 1 and highly cross-reactive with Fel d 4 (67% amino acid identity) has recently been identified in a population of cat- and dog-sensitized patients [98]. Three lipocalins from guinea pig *Cavia porcellus* (Cav p 1, Cav p 2 and Cav p 3) have also been described as major allergens [99,100]. The degree of homology between Bla g 4 and the mammalian allergens is low (approximately 15–18%) and small cross-reactivity with mammalian allergens would be expected.

Sensitization to most lipocalins occurs by inhalation, except for Bos d 5 which is β-lactoglobulin from milk, a food allergen. Lipocalins are small extra-cellular proteins present in secretions such as saliva or urine. The function of lipocalins is associated to their capacity to bind small hydrophobic ligands such as pheromones, steroids, retinoids and arachidonic acid. Bos d 5 binds palmitate and retinol, and the rodent urinary proteins Mus m 1 and Rat n 1 bind pheromones [96].

Bla g 4 is an 18 kDa lipocalin, only expressed in the adult male reproductive system, specifically in the utricles and the conglobate gland [57,101]. The allergen is involved in a male reproductive function: it is secreted with other seminal molecules during sexual activity and transferred to the female within the spermatophore during copulation [101]. The presence of Bla g 4 in the discarded spermatophore expelled by the mated female suggests that Bla g 4 may be a component of the spermatophore rather than the seminal secretions [101].

The X-ray crystal structures of Bla g 4 and a *P. americana* homolog have been published [46]. Both proteins have a typical lipocalin fold consisting of a C-terminal α-helix and a single eight-stranded (ten-stranded for Bos d 5) antiparallel β-barrel enclosing an internal cavity. The three-dimensional folding of lipocalins is conserved despite an amino acid identity as low

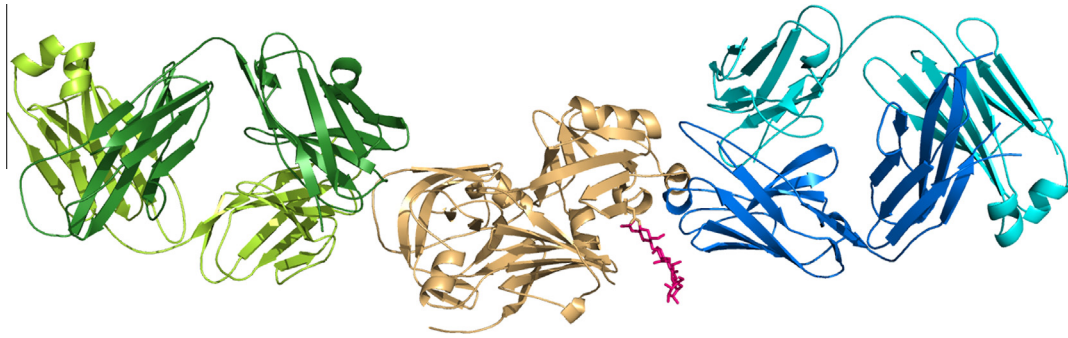


Fig. 1. Ribbon diagram of the X-ray crystal structure of rBla g 2 (gold) in complex with a Fab fragment of the mAb 4C3 (blue), superimposed to the structure of Fab' of mAb 7C11 in complex with Bla g 2 (allergen not shown). Epitopes for both mAbs are located on opposite lobes of Bla g 2: mAbs 7C11 and 4C3 bind to the N-terminal and C-terminal lobes, respectively. Antibody fragments consist of a heavy and a light chain, in dark and light green for mAb 7C11, and in marine blue and cyan for mAb 4C3, respectively. Carbohydrates involved in mAb 4C3 binding are shown in red. The figure was prepared using the software PyMOL [89].

as 20%. Bla g 4 behaves as a monomer, whereas the *P. americana* protein dimerizes in solution. Both proteins showed cross-reactivity in a Singaporean Chinese population tested, and a major IgE binding epitope unique to Per a 4 was identified on the loops at the bottom of the β -barrel [46].

6.5. Bla g 5: a glutathione transferase homolog

Bla g 5 is a relevant major cockroach allergen that belongs to the family of glutathione S-transferases (GST). The allergen protein (23 kD) shares sequence homology (42–51% identity) with the GST-2 subfamily from insects, including *Anopheles gambiae* and *Drosophila melanogaster* [63]. Recombinant Bla g 5 expressed in *E. coli* showed low enzymatic activity using the GST substrate CDNB (1-chloro-2,4-dinitrobenzene), and had a capacity to bind glutathione which is useful for purification purposes by affinity chromatography [63].

Glutathione S-transferases are very common enzymes in most forms of life and are involved in the detoxification of endogenous and xenobiotic toxic compounds. Up-regulation of GST expression in insects is associated with resistance to insecticides. Therefore, attempts to exterminate cockroaches in homes of allergic patients could lead to an undesired increase of Bla g 5 production and exposure that could contribute to worsening of allergic symptoms [63].

A Delta class glutathione S-transferase from *B. germanica* with 14% identity to Bla g 5 was reported to bind serum IgE from patients with cockroach allergy. This GST could be a cockroach allergen, but it has not yet been submitted to the WHO/IUIS Allergen Nomenclature database [102,103]. Allergens homologous to Bla g 5 are Der p 8 from dust mite and Alt a 13 from *Alternaria alternata*. Antigenic cross-reactivity has been reported between mite and cockroach GST [104].

A recent study by Santiago and co-workers revealed cross-reactive antibody responses to Bla g 5 and glutathione-S transferase from the helminth *Wuchereria bancrofti*, a major lymphatic filarial pathogen of humans [105]. Despite the fact that both proteins present low degree of amino acid identity (30%), there was extensive similarity in the N-terminal region and a similar overall fold on predicted 3-dimensional models. Interestingly, IgE, IgG, and IgG4 antibodies to both Bla g 5 and filarial GST could be detected in individuals infected with filaria, with significant correlation. In addition, mice infected with filaria developed IgE antibodies to filarial GST and showed positive skin reactivity to Bla g 5. In conjunction, these data demonstrated that helminth glutathione-S-transferase and the aeroallergen Bla g 5 share epitopes that can induce allergic cross-sensitization [105].

6.6. Cockroach allergens with homology to proteins involved in contraction: Groups 6, 7 and 8

The Group 7 of cockroach allergens comprises Bla g 7 and Per a 7. A tropomyosin from the dusky brown cockroach (*P. fuliginosa*), with 100% and 98.2% amino acid identity to the *P. americana* and *B. germanica* homologues, respectively, has also been reported [106]. Tropomyosin is a pan-allergen ubiquitously present in muscle of numerous animal species. Originally identified as a shrimp allergen, it is present in a wide number of arthropods (mollusks, arachnids, insects) and parasites [53,67–69,107–110]. IgE responses to shrimp tropomyosin have been shown to improve diagnosis of shrimp allergic patients [111,112]. The following tropomyosin allergens are reported in the WHO-IUIS list of Allergen Nomenclature: cockroach (Group 7), crab (Cha f 1, Por p 1), lobster (Hom a 1, Pan s 1), midge (Chi k 10), mite (Group 10), nematode (Asc l 3, Ani s 3), shrimp (Cra c 1, Met e 1, Lit v 1, Pan b 1, Pen a 1, Pen i 1, Pen m 1), silverfish (Lep s 1), snail (Hel a s 1), squid (Tod p 1) and tilapia fish (Ore m 4). Homology among these allergens is high (~80%), and provide the basis for antigenic cross-reactivity [113,114].

To address the issue of cross-reactivity among shrimp, cockroach, and house dust mite, Wang et al. investigated the correlation of IgE mediated sensitization to shrimp, cockroach, and dust mite in relation to allergen exposure in inner-city children [115]. Analysis of 504 serum samples from the National Cooperative Inner-City Asthma Study revealed a strong positive correlation between shrimp, cockroach, and dust mite IgE levels. Interestingly, high exposure to cockroach in the home showed significant correlation not only to higher IgE levels to cockroach, but also to higher IgE levels to shrimp. This effect of cockroach exposure was not observed for mite IgE. Therefore, in this population, having high exposure to cockroach in the home appeared to drive the immune response towards higher shrimp IgE levels [115]. It would be very interesting to establish whether the augmented IgE response to shrimp would be clinically relevant, in a way that it would pose these inner city children at a higher risk of allergic reactions upon ingestion of shrimp.

Interestingly, tropomyosins from invertebrates share much lower homology with vertebrate homologues (~55% identity) and cross-reactivity between vertebrate and invertebrate tropomyosins is absent [116]. Regardless of the degree of homology among tropomyosins, they are expected to share a similar overall fold. The three-dimensional structure of tropomyosin from some non-allergenic proteins is already known and consists of two parallel α -helical molecules wound around each other, known as coiled-coil structure.

Additional minor cockroach allergens involved in contraction have been identified that belong to Groups 6 and 8, with homology to troponin C and myosin light chain, respectively [66]. Bla g 6 comprises three isoallergens with a 14% prevalence of IgE antibodies. Troponin C is a major component in the regulation of muscle contraction. It assembles with troponin I (the inhibitory subunit) and troponin T (the tropomyosin-binding subunit) to form the troponin complex, which, in combination with tropomyosin, is located on the actin filament. Troponin C has two globular lobes of α -helices connected by a flexible linker. This linker confers molecular flexibility, which is essential for the regulatory function of troponin C. The C-lobe integrates the protein into the troponin complex, whereas the N-terminal lobe has a regulatory function, for which calcium binding to EF-hand calcium motifs is essential. An EF-hand motif is formed by a twelve amino acid binding loop flanked by two perpendicular α -helices [117]. While troponin C from vertebrate cardiac and skeletal muscle binds three and four Ca^{2+} , respectively, troponin C from arthropods (including cockroaches) and mollusks binds only two Ca^{2+} , one to each of the two terminal lobes present in the molecule. In presence of calcium, the linker is a long α -helix, and troponin I is bound to troponin C. When calcium is low, the α -helix bends 90° and releases troponin I which inhibits actomyosin ATPase by interacting with actin. Interestingly, IgE binding to Bla g 6 was proven to be calcium dependent, with the highest values of bound antibody measured when calcium was added to calcium-depleted allergen [66]. This observation indicates preferential induction of IgE production against one of the Bla g 6 molecular conformations, and shows the relevance of the allergen three-dimensional structure for generation of IgE antibodies.

Finally, Bla g 8 is a myosin light chain that shares 81–84% amino acid sequence identity with homologous proteins from the caterpillar *Lonomia obliqua*, the cricket *Gryllotalpa orientalis* and the silkworm *Bombyx mori*, and 60–66% identity to homologues from fly (*Drosophila*) and mosquito (*Anopheles* and *Aedes*) species. The prevalence of sensitization to Bla g 8 is low (14%). However, a myosin light chain from white pacific shrimp (*Litopenaeus vannamei*), Lit v 3, was cloned from a cDNA library and identified as a major allergen recognized by 55% (21/38) of shrimp allergic patients [118]. The myosin regulatory light chains are small acidic polypeptides non-covalently bound to the neck region of the myosin head, which regulate the interaction of the myosin head with actin [119]. Their regulatory function is also mediated by Ca^{2+} binding to one EF-hand motif.

6.7. Group 9: arginine kinases

Pen m 2 from black tiger shrimp (*Penaeus monodon*) was the first arginine kinase to be described as an allergen, and showed 100% reactivity by intradermal skin testing in six shrimp allergic patients using small allergen amounts ($100\ \mu\text{l}$ of 1×10^{-9} or $5 \times 10^{-9}\ \text{M}$) [120]. Subsequently, Per a 9 was identified as a major cockroach allergen and a *B. germanica* homolog was cloned. Arginine kinases are involved in the metabolism of ATP, by catalyzing the reversible transfer of the high energy phosphoryl group from ATP to arginine, which yields in addition to ADP, N-phosphoarginine, an intermediate storage and transport form of energy in a wide variety of invertebrates [120].

Additional arginine kinases reported in the WHO/IUIS official list of allergens are (in alphabetical order): Bomb m 1 from domestic silkworm *Bombyx mori*, Cra c 2 from North Sea shrimp, Der p 20 from house dust mite, Lit v 2 from the shrimp *Litopenaeus vannamei* [121] (96% identical to Pen m 2) and Plo i 1 from the Indian meal moth *Plodia interpunctella*.

Inhibition of IgE antibody binding to dust mite, cockroach, king prawn, lobster and mussel extracts by recombinant Plo i 1

suggested that arginine kinase is a cross-reactive invertebrate pan-allergen [122].

6.8. Group 10: serine proteases

The presence of proteases in cockroach extracts has been known for a long time [123]. In 2008, a serine protease was reported as a major allergen (Per a 10) of *P. americana* in an Indian population [40]. The 28 kDa protein had an IgE reactivity in 82% (37 of 45) of cockroach sensitized patients by skin tests and immunoblot. The N-terminal sequence (IVGGRPAQI) revealed similarity with mite serine protease allergens and insect trypsins. It has also been reported that the serine protease activity of Per a 10 augmented allergen-induced airway inflammation in a mouse model, and that Per a 10 biased dendritic cells towards type 2 by upregulating CD86 and inducing low IL-12 secretions [65,124]. A recombinant Per a 10 without proteolytic activity was recently expressed in *E. coli*. The Per a 10 protein sequence exhibited 27–38% similarity to the mite serine protease, and 41–52% similarity to other insect trypsins. The allergen showed potential for immunotherapy by displaying reduced IgE antibody binding and histamine release [125].

7. Cockroach allergens for diagnosis and therapy

The availability of multiple recombinant cockroach allergens opens several possibilities for developing *in vitro* tests for diagnosis of cockroach allergy. Some of them have already been outlined in this review, including ELISA, streptavidin ImmunoCAP, ImmunoCAP-ISAC, chimeric ELISA, and multiplex array assays.

In Taiwan, Chuang et al. have shown that IgE reactivity profiles were heterogeneous, in a group of 32 cockroach allergic patients [51]. Using a panel of recombinant cockroach allergens Bla g 1, Bla g 2, Bla g 4, Bla g 5, Bla g 7, and the newly identified *B. germanica* enolase, arginine kinase, and vitellogenin, the authors demonstrated substantial differences in the prevalence of IgE reactivity to each of the allergens. All patients reacted to at least one recombinant allergen on an IgE dot-blot immunoassay. The prevalence of IgE recognition was highest for Bla g 2 (63%), followed by Bla g 4 (53%), vitellogenin (47%), Bla g 1 and arginine kinase (34%), Bla g 5 and Bla g 7 (31%), and enolase (25.0%), as judged by relative densitometric indexes [51].

Skin testing using a panel of 5 recombinant allergens (rPer a 1, rPer a 7, rBla g 2, rBla g 4 and rBla g 5), was performed on 57 cockroach allergic patients with asthma and/or rhinitis living in Brazil. Twenty-four patients (42%) had positive responses to rPer a 7. Results of skin tests paralleled those of *in vitro* tests for IgE to Per a 7, with good concordance rate [12]. The frequency of sensitization to Per a 7 of 43% to 54%, considering *in vivo* and *in vitro* testing, respectively, was in keeping with previous studies in Brazil [53]. On the other hand, reactivity to the other cockroach allergens tested was remarkably low. Of the 57 patients tested, only 3 (5.3%), 4 (7%), 3 (5.3%) and 4 (7%) showed positive skin prick tests to Per a 1, Bla g 2, Bla g 4 and Bla g 5, respectively. Overall, 28/57 (49.1%) had skin tests to at least one recombinant allergen [12].

The data from Brazil showed striking differences from the results of the US study by Satinover et al. (see Section 5) [54]. In the US, IgE reactivity to rPer a 7 was very low. Even when analyzing sera of patients with co-sensitization to house dust mites, only 15/93 (16%) were positive to cockroach tropomyosin, suggesting that co-sensitization was due to concomitant exposure to dust mites and cockroach, rather than to allergenic cross-reactivity. In keeping with this, analysis carried out in Europe revealed a low frequency of IgE reactivity to mite tropomyosin (Der p 10) among mite allergic patients from various European countries. IgE to Der p 10 was

found in 6–18% of the patients [126,127]. On the other hand, analysis of sensitization profiles in a large group of 650 allergic patients in Africa revealed a 55% prevalence of IgE to mite tropomyosin [128].

It is possible that the high frequency of reactivity to cockroach tropomyosin seen in Brazil reflects cross-reactivity to mite tropomyosin, which shares 80% sequence identity to the cockroach homolog. However, co-sensitization to both tropomyosins from different origin cannot be ruled out. We hypothesize that the high frequency of sensitization to tropomyosins in Brazil and Africa may be due to cross-reactivity to tropomyosin from intestinal parasites, particularly *Ascaris lumbricoides* [68,129]. Tropomyosins from parasites including *A. lumbricoides*, filaria and *Anisakis simplex* show approximately 70% amino acid sequence identity to tropomyosins from mites and cockroach. Recombinant *A. lumbricoides* tropomyosin was shown to bind IgE from patients with asthma and/or rhinitis in 42–68% of patients from Colombia and Brazil [68,130]. IgE responses to inhaled tropomyosin allergens could be amplified or develop more promptly as a result of previous sensitization to *Ascaris* parasite tropomyosin, triggering persistent lung inflammation. In keeping with that, several reports and a meta-analysis revealed that current infection with *A. lumbricoides* was associated with a significant increase in the risk of asthma [131,132]. In affluent countries, where frequency of infections with parasites is much lower, this effect would not play a significant role.

Santiago et al. have provided further evidence indicating that parasitic infections may induce allergic responsiveness [133]. The authors have assessed the structural and immunologic relationships between *D. pteronyssinus* tropomyosin (Der p 10) and filarial tropomyosin from *Onchocerca volvulus*. Mite and filarial tropomyosins showed 72% identity in amino acid sequence, and similar predicted three-dimensional structures. The prevalence of IgE and IgG to Der p 10 was increased in filaria-infected individuals, with a strong correlation of serum levels of IgE, IgG and IgG4 to Der p 10 and filarial tropomyosin. In addition, primates experimentally infected with filaria *Loa loa* produced IgE that cross-reacted with Der p 10 [133]. In keeping with a possible mechanism by which parasites could regulate allergic reactivity through cross-reactive antibody responses, several parasite molecules have been reported to have homologues in allergen families other than tropomyosins, including lipocalins, EF-hand proteins, cupin superfamily, profilins and glutathione-S-transferases [134].

In current clinical practice, diagnosis of cockroach allergy is performed by skin testing and/or measurement of specific IgE to cockroach, using crude extracts. Cockroach extracts manufactured in the United States for allergy diagnosis show variation in levels of major allergens Bla g 1 and Bla g 2 of up to sevenfold [135]. Slater et al. have shown that the mean potency of three US German cockroach extracts was 3300 BAU/mL, using the intradermal D50 method, whereas standardized mite, cat or grass extracts contain typically 5000–100,000 BAU/mL [136]. Standardization of German cockroach extracts is currently being pursued by the US Food and Drug Administration.

Immunotherapy is also practiced using crude, non-standardized cockroach extracts, and its effectiveness has not been established. There have been very few reports of immunotherapy for cockroach allergy [137–139]. In a study carried out by Kang et al. in 1988, involving a small number of patients, immunotherapy with cockroach extract resulted in beneficial changes in immunological and clinical parameters, including symptoms and medication use, after 5 years of treatment [137]. Decrease in nasal symptoms and increase in cockroach specific IgG levels, accompanied by decrease in serum levels of IL-2, IL-4 and IL-4 receptor were found following 3 years of immunotherapy with cockroach extract [138]. More recently, a double-blind, placebo controlled cockroach immunotherapy trial from India has been reported [139]. Immunotherapy was

carried out using an in-house cockroach extract, prepared under good manufacturing practice conditions, and 50 cockroach allergic patients with asthma and/or rhinitis were enrolled, of whom 42 (28 active treatment, 18 placebo) completed the first year of the study. Results at one year, while patients were still on build-up phase, showed significant improvement in clinical scores and bronchial hyper-reactivity, and increase in cockroach specific IgG4. Unfortunately, the study did not include placebo-treated subjects in its second year, and only 12 patients in the active treatment group provided samples for analysis at the end of the two-year follow up. Other limitations of this study include little information on how symptom and medication scores were developed, and lack of information on adverse reactions to immunotherapy [139]. Although these studies suggest that cockroach immunotherapy may be effective, further randomized, controlled clinical trials, with larger number of patients and longer follow up, and using well-characterized cockroach extracts, will be required to establish efficacy and safety of this form of treatment. Studies on subcutaneous and sublingual immunotherapy with German cockroach extract have been carried out in the United States [140], according to the ClinicalTrials.gov registry by the National Institutes of Health. However, results of these studies have not yet been reported.

Purified cockroach allergens have been used in experimental mouse models for prophylaxis or treatment of cockroach-induced allergic lung inflammation [141,142]. A DNA vaccine-encoding Bla g 2 was shown to be effective in suppressing airway inflammation and inducing a Th1 response, while suppressing a Th2 reaction, when used prior to sensitization in mice [141]. Cockroach sensitized mice, treated with liposome-entrapped natural Per a 9 (arginine kinase) via the intranasal route, presented significantly reduced respiratory tract inflammation after provocation. In addition, these mice also had an immune deviation from the allergic Th2 response to Th1 and Treg responses, as indicated by the lung cytokine profiles [142].

It could be envisioned that recombinant allergens would be able to lead us to a better understanding of the development of cockroach allergy and of the association with severity of asthma, through mechanistic studies. T cell responses to peptides derived from major allergens of the German cockroach have been recently investigated [143]. Bioinformatic strategies predicted binding of Bla g 1, 2, 4, 5, 6, and 7 peptides to HLA-DR, -DP, and -DQ molecules. In conjunction with PBMC responses from 30 allergic patients, 25 T cell epitopes were identified, including 5 epitopes comprising over half of the response. Bla g 5 was the dominant allergen (65% of the response), and induced both IL-5 and IFN- γ responses, whereas Bla g 6 induced mostly IL-5, and Bla g 2 induced only IFN- γ . These results indicated that responses to *B. germanica* allergens were independently regulated, and suggested a critical role for the allergen itself. Comparison of IgE and T cell responses to particular allergens in the same donor revealed presence of IgE titers in absence of T cell responses, suggesting that unlinked T cell–B cell help could support development of IgE responses. In the same study, subcutaneous immunotherapy for 6 month with cockroach extract in a subgroup of nine patients resulted in down-regulation of IL-5, which was not associated with development of IFN- γ or IL-10 responses to any of the peptides. Therefore, results of this study indicated that T cell responses to *B. germanica* allergens appear uncorrelated with IgE responses [143].

More desirable, recombinant allergens could be used for diagnostic purposes, by either performing skin testing or detecting specific IgE antibodies in sera, leading to increased sensitivity and specificity in the diagnosis of cockroach allergy. Ultimately, recombinant allergens could be used in clinical trials for immunotherapy of cockroach allergic patients, particularly those at a higher risk for more severe disease such as children and young adults living in

inner-city environments in the US. However, further analyses are needed to better understand the relative importance of different cockroach allergens in a wider range of populations, which will provide insights into cockroach allergen sensitization depending on allergen exposure, geographic location and genetic background.

8. Concluding remarks

Cockroach allergy is a risk factor for emergency room admissions with asthma. Chronic exposure to low levels of allergens indoors leads to sensitization in susceptible individuals, and subsequent exacerbation of symptoms. Although abatement measures to reduce cockroach infestation, and therefore exposure to cockroach allergens, can contribute to improvement of allergic symptoms, they are difficult to maintain, especially in low socio-economic inner-city areas. Ten Groups of cockroach allergens are presently described at the WHO/IUIS Allergen Nomenclature database. The advent of molecular cloning and expression of recombinant allergens has led to an increased understanding of the function and structure of cockroach allergens, and the contribution of these intrinsic properties to the development of disease. The structure of allergen–antibody complexes solved by X-ray crystallography has revealed allergen function and mechanisms of antibody recognition for the design of therapeutic hypoallergens. Despite the importance of cockroach allergy, the number of reports on cockroach allergen immunotherapy is very limited. Therefore, knowledge of the intrinsic properties of cockroach allergens and mechanisms of disease will allow the development of new approaches to therapy.

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