

Immunological Aspects of the Immune Response Induced by Mosquito Allergens

José Fernando Cantillo^{a, b} Enrique Fernández-Caldas^{c, d} Leonardo Puerta^a

^aInstitute for Immunological Research, University of Cartagena, and ^bCorporación Universitaria Rafael Núñez, Cartagena, Colombia; ^cInmunotek S.L., Madrid, Spain; ^dDivision of Allergy and Immunology, College of Medicine, University of South Florida, Tampa, Fla., USA

Key Words

Mosquito allergy · *Aedes aegypti* · Immunoglobulin E · Recombinant allergens · Cross-reactivity

Abstract

Allergies caused by mosquito bites may produce local or systemic reactions. The inhalation of mosquito allergens may also cause asthma and/or allergic rhinoconjunctivitis in sensitized individuals. The mechanisms implicated in the development of these immune responses involve IgE antibodies, different subtypes of IgG and proinflammatory cytokines as well as basophils, eosinophils and mast cells. Several allergenic components have been identified in the saliva and bodies of mosquitoes and some of these are present in different mosquito species. The most common species implicated in allergic reactions belong to the genera *Aedes*, *Culex* and *Anopheles*. Several *Aedes aegypti* allergens have been cloned and sequenced. The recombinant molecules show IgE reactivity similar to that of the native allergens, making them good candidates for the diagnosis of mosquito allergies. Allergen-specific immunotherapy with mosquito extracts induces a protective response characterized by a decreased production of IgE antibodies, increased IgG levels, a reduction in the severity of cutaneous and respiratory symptoms and the need for medication. The aims of this review are to summarize the progress made in the characterization

of mosquito allergens and discuss the types of immune responses induced by mosquito bites and the inhalation of mosquito allergens in atopic individuals.

© 2015 S. Karger AG, Basel

Introduction

Mosquitoes are insects belonging to the order Diptera and members of a family of the nematocerid flies Culicidae (from the Latin *culex*, genitive *culicis*, meaning ‘midge’ or ‘gnat’) [1]. The word ‘mosquito’ (formed by ‘mosca’ and the diminutive ‘ito’) is from the Spanish or Portuguese for ‘little fly’ [2]. Mosquitoes resemble crane flies (family Tipulidae) and chironomid flies (family Chironomidae). The females of many species of mosquitoes are blood-eating pests and dangerous vectors of disease whereas members of the similar-looking Chironomidae and Tipulidae are not.

At least 14 orders of arthropods, containing over 400 different genera and more than 15,000 species, evolved to feed on blood from warm vertebrate animals [3]. Three subfamilies are recognized within the family Culicidae, namely Toxorhynchitinae, Anophelinae and Culicinae. Toxorhynchitinae includes only one genus, while Anophelinae consists of nearly 400 species. More than 2,600 mosquito species belong to the subfamily Culicinae. More

than 40 genera and 3,000 species of mosquitoes are distributed worldwide (table 1) [4]. Some of these species are more clinically relevant than others because they affect humans, directly or indirectly. These species include, but are not limited to, *Aedes aegypti*, *Aedes vexans* and *Culex quinquefasciatus* [5–7]. Adult female mosquitoes require blood-feeding to produce eggs. In this process, they bite and inject their saliva before sucking their victim's blood [8]. Mosquito saliva contains various substances like lysozymes, antibacterial glucosidases, anticoagulants, antiplatelet aggregating factors and vasodilators [9–13]. It also contains allergens that may induce local cutaneous reactions and, in some cases, a systemic response in atopic individuals [14–17]. Allergens derived from the emanations and detritus of mosquitoes are also present in the air and in house dust, and may be inhaled and induce various immune responses [18, 19].

The term 'mosquito allergy' is used to describe a series of signs and symptoms caused by mosquito bites. Individuals may experience a variable degree of symptoms after a mosquito bite consisting of unusual large local ecchymotic and vesiculated reactions and, in some cases, systemic reactions, such as generalized urticaria, angioedema or wheezing and even anaphylaxis [20–23]. However, it has also been shown that mosquito debris remains suspended in the air and produces an allergic respiratory response similar to that caused by other airborne allergens, such as those from the dust mite and cockroach [18, 24, 25].

Allergies caused by mosquitoes have a negative impact on the affected population, on their quality of life and, in some cases, even put their lives at risk. Although the general opinion is that mosquito allergy may be highly prevalent, there are only a few epidemiological studies supporting this perception. In a cross-sectional study conducted in Mexico City, 82% of the patients who were admitted to the allergy service reported that they were allergic to mosquito bites; however, only 2.5% of them showed positive skin reactions to this insect [26]. In another study, conducted in India, it was shown that 47% of the population with asthma and/or allergic rhinitis were sensitized to mosquito allergens, as determined by skin-prick tests, serum IgE antibodies and bronchial provocation tests with whole mosquito body extracts [25]. There is no epidemiological data about mosquito allergy in South America, where several mosquito species and mosquito-borne diseases are highly prevalent.

Mosquito sensitization may also be associated with other diseases. Children and individuals with primary or

secondary immunodeficiency are most at risk for mosquito-bite allergy, especially those living in areas heavily exposed to mosquitoes and their allergens [23, 27, 28]. Several cases have been described in which sensitization to mosquito bites is accompanied with natural killer (NK) cell lymphoproliferative disorders, the presence of T cells infected with Epstein-Barr virus or lymphocytosis associated with HIV infection [29–32]. The study by Asada et al. [33] demonstrated that mosquito saliva induces lymphoproliferation, which, in turn, increases the expression of an oncogene in NK cells, which apparently plays an important role in the etiology of mosquito allergy. It has been shown that the inflammatory and/or allergic response induced by mosquito bites, especially *Anopheles* spp., favors infection with *Plasmodium* spp., thereby facilitating the occurrence of malaria [34, 35]. Therefore, mosquito-sensitized individuals seem to be at a greater risk of contracting malaria.

Allergy to house dust mites is one of the most important risk factors for asthma in tropical regions. The main species involved are *Blomia tropicalis* and *Dermatophagoides pteronyssinus* [36–38]. Mosquitoes are abundant in these latitudes, since the climatic conditions of tropical regions favor the life cycle and proliferation of mosquito species [39–42]; this fact might favor high exposure to their allergens. Mosquitoes, like mites, may also be an important environmental factor that induces or exacerbates allergic diseases. Moreover, studies on mosquitoes, mites and cockroaches indicate that these arthropods have some allergens in common [43–46]. These allergens have not been investigated and identified yet, despite the fact that cross-reactivity between mites and mosquito may play a significant role in the prevalence and clinical manifestations of allergic respiratory and dermatological diseases.

Immunological Basis of the Allergic Reactions Produced by Mosquitoes

Symptoms and Clinical Stages of Reactivity

Mosquito bites produce a local reaction consisting of a rash and heat, with the subsequent appearance of a wheal and flare, and itching in nonatopic individuals. The term 'mosquito allergy' refers to reactions which cause severe or atypical (ecchymotic or vesiculated) local or systemic reactions, such as anaphylaxis, angioedema, generalized urticaria or wheezing. Four types of reactions occur as the consequence of a mosquito bite: (1) local reactions (immediate and delayed), (2) severe, delayed reactions

Table 1. Taxonomical classification and distribution of the main mosquito species associated with mosquito allergy (including those to which sensitization has been demonstrated in vivo/in vitro)

Subfamily	Genera	Subgenera, n	Species, n	Distribution	Species associated with mosquito allergy
Anophelinae	<i>Anopheles</i> <i>Bironella</i> <i>Chagasia</i>	7 3 –	455 8 4	cosmopolitan Australasian neotropical	<i>A. stephensi</i> , <i>A. minimus</i> , <i>A. sinensis</i>
<i>Culicinae</i> Aedeomyiini	<i>Aedeomyia</i>	2	6	Afrotropical, Australasian, Oriental, neotropical	
Aedini	<i>Aedes</i>	23	363	Old World, Nearctic	
	<i>Argimères</i>	2	58	Australasian, Oriental	
	<i>Ayurakitia</i>	–	2	Oriental	<i>A. aegypti</i> , <i>A. vexans</i> , <i>A. communis</i> , <i>A. togoi</i> , <i>A. albopictus</i> , <i>A. triseriatus</i>
	<i>Borichinda</i>	–	1	Oriental	
	<i>Eretmapodites</i>	–	48	Afrotropical	
	<i>Haemagogus</i>	2	28	principally neotropical	
	<i>Heizmannia</i>	2	39	Oriental	
	<i>Ochlerotatus</i>	22	550	cosmopolitan	<i>O. triseriatus</i> , <i>O. hendersoni</i>
	<i>Opifex</i>	–	1	New Zealand	
	<i>Psorophora</i>	3	48	New World	
	<i>Udaya</i>	–	3	Oriental	
	<i>Verrallina</i>	3	95	Principally Australasian, Oriental	
	<i>Zeugomyia</i>	–	4	Oriental	
Culicini	<i>Culex</i>	23	763	cosmopolitan	<i>C. quinquefasciatus</i> , <i>C. tritaeniorhynchus</i> , <i>C. pipiens</i> , <i>C. pipiens pallens</i> , <i>C. tarsalis</i>
	<i>Deinocerites</i>	–	18	principally neotropical	
	<i>Galindomyia</i>	–	1	neotropical	
	<i>Lutzia</i>	3	7	Afrotropical, Australasian, Oriental, neotropical, Palearctic oriental	
Culisetini	<i>Culiseta</i>	7	37	New World, Nearctic	<i>C. inornata</i>
Ficalbiini	<i>Ficalbia</i> <i>Mimomyia</i>	– 3	8 44	Afrotropical, Oriental Afrotropical, Australasian, Oriental	
Hodgesiini	<i>Hodgesia</i>	–	11	Afrotropical, Australasian, Oriental	
Mansoniini	<i>Coquillettidia</i> <i>Mansonia</i>	3 2	57 23	Old World, Neotropical Old World, Neotropical	
Orthopodomyiini	<i>Orthopodomyia</i>	–	38	Afrotropical, nearctic, neotropical, Oriental, palearctic	
Sabethini	<i>Isotomyia</i> <i>Johnbelkinia</i> <i>Kimia</i> <i>Limatus</i>	– – – –	4 3 5 8	neotropical neotropical Oriental neotropical	

Table 1. (continued)

Subfamily	Genera	Subgenera, n	Species, n	Distribution	Species associated with mosquito allergy
	<i>Malaya</i>	-	12	Afrotropical, Australasian, Oriental	
	<i>Maorigoeldia</i>	-	1	New Zealand	
	<i>Onirion</i>	-	7	neotropical	
	<i>Runchomyia</i>	2	7	neotropical	
	<i>Sabethes</i>	5	38	neotropical	
	<i>Shannoniana</i>	-	3	neotropical	
	<i>Topomyia</i>	2	54	principally Oriental	
	<i>Trichoprosopon</i>	-	13	neotropical	
	<i>Tripteroides</i>	5	122	principally Australasian, Oriental	
	<i>Wyeomyia</i>	15	140	Principally neotropical	
Toxorhynchitini	<i>Toxorhynchites</i>	4	88	Afrotropical, Australasian, neotropical, palearctic Oriental, Oriental	
Uranotaeniini	<i>Uranotaenia</i>	2	265	Afrotropical, Australasian, Oriental, neotropical	
This table has been modified from data in Harbach [4].					

with scar formation (via a cell-mediated mechanism), (3) exaggerated local reactions, and (4) systemic anaphylaxis [47]. Local cutaneous reactions consist of immediate wheals and flares peaking at 20 min and delayed pruritic indurated papules peaking at 24–36 h [48, 49]. Large local reactions consist of itchy red, warm swellings appearing with minutes, itchy papules and ecchymotic, vesiculated, blistering, bullous or even Arthus-type reactions, appearing 2–6 h after the bites [20, 23, 50].

Sensitization may occur after the first exposure to a mosquito bite. Subsequent bites may induce the characteristic signs and symptoms of an allergic reaction. Based on clinical observations, the natural history of sensitization and desensitization to mosquito bites has been classified into 5 well-defined clinical stages: (1) no reaction after the initial bite in individuals who have never been bitten, (2) delayed cutaneous lesions appear after approximately 24 h in individuals after subsequent bites, (3) wheals appear within approximately 15 min in individuals after repeated bites, (4) no delayed reactions occur and only immediate wheals are noted in individuals after further exposures to mosquito bites, and (5) there is a loss of the immediate reaction in individuals who are repeatedly exposed to huge numbers of mosquito bites [51–53].

Evidence suggests that mosquitoes might also induce allergic respiratory symptoms through the inhalation of suspended allergens that derive from their bodies and emanations. The available evidence and similar findings with regard to other flying insects suggest that asthma and allergic rhinitis might be exacerbated by the inhalation of mosquito allergens, in a similar way to other airborne allergens derived from arthropods such as mites, moths [54], bees [55] and cockroaches [56].

Humoral and Cellular Response to Mosquito Bites

Immunological changes that occur during the process of sensitization and desensitization have been studied in humans and in animal models, and involve the humoral as well as the cellular components of the immune response. In a prospective study [48] on the natural course of the allergic response against mosquito bites, a nonallergic individual was exposed to 100 bites of *C. quinquefasciatus* every 2 weeks for 10 months. The levels of specific IgE and IgG antibodies increased steadily with each exposure. Skin tests with a mosquito extract were positive, and the size of the wheal correlated with IgE and IgG antibody levels. However, after 21 weeks of exposure, the levels of specific antibodies to the extract decreased. Although these results demonstrate that an individual may

be desensitized to mosquito bites, in real life, such individuals try to avoid contact and thus reduce the number of bites, thereby requiring more time for desensitization. In a population of children from Winnipeg, Man., Canada, high levels of specific IgE and IgG occurred in the first 6 months, but had decreased by adolescence, suggesting that these children were desensitized naturally by exposure [57]. Kulthanan et al. [21] showed the required interval between sensitization and desensitization in a population in Thailand to be approximately 4 years.

The participation of specific IgE in mosquito allergy has been demonstrated by immediate skin reactivity induced by the passive transfer of specific IgE antibodies [58]. These antibodies were associated with local and systemic, and immediate or delayed reactions. It has been demonstrated that specific IgE levels are higher in the serum of individuals allergic to at least 1 of 10 mosquito species distributed worldwide than those in nonallergic individuals [44]. Brummer-Korvenkontio et al. [22] showed that individuals sensitized to *Aedes communis* with immediate and delayed reactivity had high levels of specific IgE and IgG4 to *A. communis*. Peng et al. [14] showed that individuals sensitized to mosquito bites who experienced acute systemic reactions had higher specific IgE levels than nonallergic individuals. In these cases, there was also a positive and significant correlation between the size of the sting wheals and the levels of serum antibodies. It seems that IgG antibodies may play a role in immediate local reactions, but not in systemic reactions. A study by Reunala et al. [59] demonstrated that between 82–90% of children in Finland, Kenya and Mexico had specific IgE antibodies against *A. communis* and *A. aegypti*, with the presence of IgG4 antibodies in the children being 85, 41 and 20%, respectively. The titers of specific antibodies were associated with the degree of exposure. It was demonstrated that in the early allergic response caused by mosquito bites, in addition to the levels of the antibodies, those of histamine and leukotriene C4 also increased [60]. In a mouse model of mosquito allergy, *A. aegypti* induced a Th2 immune response with immediate and late reactions and higher levels of IgE and IgG1 antibodies compared to mice that were nonsensitized. A higher lymphoproliferation was also observed in sensitized mice treated with mosquito extracts [61].

Mast cells may also play a role in allergic symptoms caused by mosquito bites. Demeure et al. [62] showed that the bites of *Anopheles stephensi* activated mast cell degranulation in healthy mice; this induced changes in vascular permeability, the extravasation of fluid and the recruitment of neutrophils at the bite site. This response

was accompanied by hyperplasia of the lymph nodes and the recruitment of dendritic cells, B lymphocytes, macrophages and T lymphocytes. These effects were not observed in mast cell-deficient animals, unless they were reconstituted with mast cells. An important finding in this study was that the in vitro degranulation of mast cells occurs in the absence of IgE, suggesting that the saliva may be responsible for the inflammatory response.

Other Manifestations of Mosquito Allergy

Papular urticaria may also appear as a hypersensitivity reaction after mosquito bites, or after the bites of other arthropods. The exact immune mechanisms behind papular urticaria are unknown, but some evidence support the involvement of type I, III and IV hypersensitivity reactions, and therefore the participation of IgE and IgG antibodies. Abdel-Naser et al. [63] showed that a group of patients with papular urticaria had specific IgG against *Culex pipiens* antigens. The levels of these antibodies correlated with the size of both immediate and delayed skin reactions and specific IgE levels [64]. Immunoglobulin and complement depositions have also been detected in the skin, suggesting that these participate in the mechanism of papular urticaria lesions [65, 66].

In some cases, the mosquito bite may also induce a strong reaction involving an extensive part of the body such as the face, hands, arms, feet or legs. These reactions are often misdiagnosed as cellulitis. However, they appear a few hours after the mosquito bites, which makes it unlikely to be due to a bacterial infection. Simons and Peng named this clinical disorder Skeeter syndrome [20].

Mosquito Allergens

It has been suggested that there may be differences in the allergenic composition of mosquito saliva, salivary glands and whole body extracts. Mosquito saliva contains allergens that sensitize atopic individuals [64]. Sixteen IgE-reactive proteins with a molecular weight of between 16 and 95 kDa have been detected in the saliva and salivary glands of 10 different mosquito species distributed worldwide [44]. When analyzed with sera from allergic individuals, allergens in the saliva of *C. quinquefasciatus* with a molecular weight of 35.5, 32.5 and 22.5 kDa showed a frequency of IgE reactivity of 41, 28 and 24%, respectively [67]. Fourteen IgE reactivity fractions with a molecular weight of between 23 and 93 kDa were identified using sera from allergic patients in extracts from the salivary glands of *Aedes togoi*, *Culex tritaeniorhynchus* and *C. pip-*

iens pallens. In addition, mice immunized with these extracts produced mainly IgG1 that recognized bands of 31, 37 and 57.5 kDa [17], respectively, suggesting that these antigens could play a protective immune response.

Several studies have shown that mosquito saliva is not the only source of allergens. In fact, extracts used for allergen-specific immunotherapy and diagnosis are produced from saliva, salivary glands or whole mosquito bodies. Although these preparations have different biological activities, all are useful for the diagnosis of allergic individuals [68]. In Yazd, Iran, a subtropical city with a warm, dry climate, 32.6% of 95 patients with allergic rhinitis had a positive skin test to whole-body mosquito extracts. The frequency of reactivity to mould, cockroach and mite extracts was lower [24]. These results suggest that the antigens contained in mosquito bodies are important environmental factors associated with allergic rhinitis in this region. An extract prepared from airborne particles collected in the homes of mosquito-allergic patients could block the binding of IgE antibodies to the antigens contained in a whole-body extract of *C. quinquefasciatus* [18]. This study proposes that mosquito allergens are present in the environment and that they retain their immunological properties, as shown in ELISA inhibition experiments. When inhaled, these allergens can induce an allergic response in atopic individuals sensitized to mosquito bites. The clinical manifestations of this response are different from those caused by insect stings [69]. Similar findings have been described in south-eastern Minnesota, where large amounts of outdoor *Lepidoptera* allergens have been detected, suggesting that the inhalation of insect debris could play an important role in the ethiopathogenesis of allergic respiratory diseases [54]. Other biting insects such as fleas are also a source of indoor allergens, but their role as inducers of allergic diseases is less known [70]. Mosquito saliva primarily induces skin reactions, while the antigens that are suspended in the air may induce respiratory symptoms. Therefore, it is important to identify and characterize the allergens associated with these different clinical manifestations, in order to design better strategies for diagnosis and treatment. Allergen cross-reactivity must also be fully delineated to the contribution of species-specific or cross-reacting allergens.

We have demonstrated that the sera from mite-allergic individuals contain specific IgE antibodies that react with more than 10 allergens from an *A. aegypti* extract [71]. Although the clinical relevance of these allergens in allergic symptoms is unknown, the results suggest that mosquitoes are a source of aeroallergens, which may play a role in allergic respiratory diseases. Wongkamchai et al.

[19] reported the presence of 16 allergens in the saliva, 6 in the salivary glands and 2 in whole-body extract of 4 mosquito species. These allergens are not common to all three sites, suggesting that allergic symptoms are related to the route of exposure (i.e. cutaneous or respiratory) and are associated with unique allergens. It is still necessary to further investigate if the allergens present in the saliva can induce respiratory symptoms.

Although several allergens have been identified in mosquito extracts, only a few from the saliva have been characterized in terms of their role in the allergic response. Most reported mosquito allergens belong to *A. aegypti* spp. (table 2) (see <http://www.allergome.org> and <http://www.allergen.org>).

Characterized Allergens

Aed a 1

This allergen shows homology with apyrase (ATP diphosphohydrolase) and has a molecular weight of 68 kDa. It interferes with platelet aggregation in human blood by hydrolyzing ADP and ATP released by the platelets and other cells [72]. It also has homology with members of a family of enzymes with 5'-nucleotidase activity, also present in humans and rats [73]. The serum of mosquito-allergic individuals may have specific IgE against this apyrase [44]. The truncated Aed a 1 (residues 150–562) was found to react with serum specific IgE and IgG antibodies of allergic individuals, suggesting that this fragment contains B cell epitopes [74]. In another study, 9 of 31 sensitized individuals (29%) had a positive skin test to rAed a 1 [15]. Rabbits immunized with Aed a 1 produced polyclonal sera that reacted with allergens in the saliva extracts of *A. vexans* and *Aedes albopictus* [44]. Apyrase activity was detected in the salivary glands of *Ochlerotatus triseriatus* and *Ochlerotatus hendersoni* [75], suggesting the presence of homologous allergens to Aed a 1. Because apyrase from mosquitoes inhibits platelet aggregation induced by collagen and thrombin, it has been suggested that it could be of therapeutic use in treating thrombosis [9]. It seems that Aed a 1 is expressed only in the salivary glands, which suggests that individuals may come into contact with this molecule through mosquito bites. However, it has still to be established whether this allergen can be detected in the environment and if it is capable of inducing sensitization through inhalation.

Aed a 2

This allergen belongs to the family of proteins called D7, which are highly conserved in insects that require blood for feeding and reproduction and are released in

Table 2. Reported *A. aegypti* allergens

Allergen	Molecular weight, kDa	Biological function	Amino acids, n	Frequency of reactivity ^a (patients, n/% positives)		References
				IgE	antigenic challenge	
Aed a 1	68	antiplatelet aggregant (apyrase)	562	–	31/29; 28/43	[15, 44, 74–76, 80]
Aed a 2	37	transport of hydrophobic molecules (pheromone and odorant)	321	21/42.8	28/11	[16, 44, 78–80]
Aed a 3	30	antiplatelet aggregant (aegyptin)	273	–	28/32	[81, 82]
Aed a 4	67	not described	579	25/36	–	[83]
Aed a 7	32.3	muscle contraction (tropomyosin)	284	–	–	see Allergome

Other mosquito species may also contain homologous allergens, as described in the text.

^a Based on serology (IgE) or skin test (antigenic challenge).

the saliva just after piercing the skin. Aed a 2 contains an N-terminal and a C-terminal domain, capable of binding to leukotrienes and biogenic amines that are released by an individual as a mechanism of defense against the mosquito bite [76]. The gene encoding this protein was first identified in a cDNA library constructed from the salivary glands of *A. aegypti*. Northern blot assays and in situ hybridization showed that the mRNA of the protein is only present in the salivary glands of female mosquitoes, suggesting that it plays an important role in blood feeding [77]. This molecule has been cloned and obtained as a recombinant protein by expression in insect cells infected with baculovirus. It retains the ability to bind to specific human IgE antibodies and induces skin reactivity in a similar way to the natural protein [78]. Mice immunized with rAed a 2 produced IgG1 and IgE antibodies capable of binding to the recombinant and natural allergens. Sensitized mice showed a positive skin test to the recombinant allergen, which suggests that this protein induces a Th2 response, characteristic of an allergic reaction. Aed a 2 cross-reacts with proteins from other species of *Aedes* (*A. vexans*, *A. albopictus*, *A. communis* and *A. togoi*), *C. quinquefasciatus* and *O. triseriatus* [44]. The saliva of *C. quinquefasciatus* contains allergens homologous to Aed a 2 [79]. These allergens are homologous with the family of proteins D7 and show high structural homology with the protein THP12 of the hemolymph of *Tenebrio molitor*, which is part of the family of pheromone-binding proteins and odorants that participate in the transport of hydrophobic molecules [80]. It seems that Aed a 2 should be included in preparations for

the diagnosis and immunotherapy of mosquito allergy, since it has a significant frequency of IgE reactivity [44] and may participate in cross-reactivity among various species. Because recombinant Aed a 2 is biologically and immunologically similar to the native purified allergen, it could be used in the diagnosis and immunotherapy of mosquito allergies as well. This would overcome the difficulty of obtaining this molecule from the saliva. Aed a 1 and Aed a 2 seem to be expressed only in mosquito salivary glands.

Additional Allergens

The allergens Aed a 3 and Aed a 4 have been obtained by molecular cloning and expression in insect cells infected by baculovirus. Aed 3 is expressed as a 30-kDa protein which binds specifically to collagen. It prevents the interaction of collagen with physiological ligands, such as platelet glycoprotein IV, integrin $\alpha 2\beta 1$ and von Willebrand factor, blocking platelet aggregation induced by collagen [81]. This property suggests its potential use as an anti-thrombotic drug. It has been suggested that this allergen may also be valuable for the diagnosis of mosquito allergy. It has been shown that a mixture of rAed a 1, rAed a 2 and rAed a 3 can identify approximately 65% of mosquito-allergic patients [82]. It has been shown that 36% of *A. aegypti*-allergic individuals have specific IgE against Aed a 4, suggesting that its inclusion in vaccine preparations might be desirable [83]. Aed a 7 is recorded in the Allergome platform, as an allergen with homology to tropomyosin. However, no further information is currently available about its biological and structural properties.

Allergen Cross-Reactivity

Allergen cross-reactivity occurs when IgE antibodies induced by exposure to an allergen react with a different protein with structural homology to the antibody-inducing allergen. This phenomenon is common among allergens from mites and other arthropods [84, 85]. The sera from individuals allergic to mosquito bites react with several common proteins present in the extracts of *C. quinquefasciatus*, *A. aegypti*, *A. albopictus* and *Anopheles minimus*, with molecular weights between 35.5 and 75.4 kDa [19]. These results suggest that exposure to 1 species may induce sensitization to other species to which the individual has not been previously exposed. In the province of Manitoba in Canada, the most prevalent mosquito species and the main sensitizer is *A. vexans*. Individuals allergic to *A. vexans* also show skin test reactivity to 5 other species not reported in Manitoba.

Mosquito-allergic individuals in Shanghai in China also have specific IgE against *A. vexans* allergens, despite the fact that this species has not been reported in this region [43, 44]. It can be speculated that, in these cases, allergen-specific immunotherapy with allergen extracts prepared from a single mosquito species might be sufficient to confer protection against other species. However, this should be carefully analyzed because species-specific allergens may exist and participate in the sensitization process. Vaccine preparations lacking species-specific allergens might thus be ineffective. A component-based molecular diagnosis would be of great value in these cases.

Rabbits immunized with rAed a 1 produce serum antibodies that react with extracts from *A. vexans* and *A. albopictus* [44], indicating that these 2 species have homolog allergens to that which is present in *A. aegypti*. In the salivary gland of *O. triseriatus* and *O. hendersoni*, a protein with apyrase activity has been detected; this might be a homolog of Aed a 1 [75]. Serum from rabbits immunized with rAed a 2 also reacted with extracts of *C. quinquefasciatus*, *O. triseriatus* and several species of *Aedes* [16, 44], indicating the participation of this allergen in the cross-reactivity among these 3 species.

C. quinquefasciatus, *A. aegypti* and *A. stephensi* express D7 family proteins; 73.1% of sera with IgE reactivity to *C. quinquefasciatus* also have specific IgE antibodies to whole-body extracts of *A. aegypti* and *A. stephensi* [80]. The D7 family is highly conserved in many species of blood-sucking insects and mites (ticks), so it is possible that cross-reactivity among these species may involve this protein family.

Studies of cross-reactivity between mosquitoes and wasps, dust mites, cockroaches and shrimp are scarce. However, some individuals develop the so-called 'wasp/mosquito syndrome', which involves a cross-reactivity phenomenon between these species. Individuals afflicted by this syndrome have specific IgE against a 44-kDa hyaluronidase in mosquitoes and wasps [86]. The existence of cross-reactivity between chironomids and mosquitoes, based on the results of positive skin tests to *C. pipiens* and serum IgE reactivity to *A. communis*, has also been suggested in individuals allergic to chironomids [45]. A study conducted in Reykjavik, Iceland, showed that most individuals sensitized to *D. pteronyssinus* also had specific IgE against mites (*Lepidoglyphus destructor*), shrimps and, to a lesser extent, cockroaches and mosquitoes [87]. There were virtually no mites in the dust samples collected from mattresses in the homes of these individuals [46], indicating an absence of exposure to mite allergens. The sensitization to mites in this population could therefore be attributed to exposure to cross-reactive allergens from mosquitoes or other arthropods.

Cross-allergenicity has important clinical and epidemiological implications, such as polysensitization to various sources of allergens in the same or different taxonomic groups. To circumvent this problem, a better diagnosis could be achieved by identifying the specific molecules involved in the allergic response, which may be performed using highly purified recombinant or natural allergens in order to define the IgE-binding profile of each patient. Component-resolved diagnosis would allow a component-resolved immunotherapy, in which only the allergens involved in sensitization could be administered and not irrelevant allergens or nonallergenic molecules [88].

Sensitization to arthropods seems to be a predisposing factor for the onset of cutaneous and allergic respiratory responses to mosquitoes. Cross-reactivity among mosquitoes and other arthropods has been suggested. However, the main allergens involved here have not been identified. In the tropics, mosquitoes and the mite species *D. pteronyssinus* and *B. tropicalis* are very common. Therefore, it is probable that cross-reactivity between these arthropods may have an impact on the prevalence and manifestations of allergic diseases. We have shown that 40% of allergic individuals from the tropical Caribbean island, Martinique, have specific IgE against *A. aegypti*. Furthermore, ELISA inhibition assays showed that *A. aegypti* contains allergens that cross-react with *D. pteronyssinus* allergens [89]. Two tropomyosin isoallergens

(Uniprot code: Q17H75 and Q17H80) of 32 kDa purified from a whole-body mosquito extract reacted with 33% of the mosquito-positive sera. They also showed cross-reactivity with the shrimp allergens: nPen m 1 and rLit v 1 [71]. These results confirm that there is a high frequency of sensitization to *A. aegypti* in mite- and shrimp-allergic individuals in Martinique. Species-specific as well as cross-reactive allergens, such as tropomyosin, are involved in IgE sensitization.

Allergen-Specific Immunotherapy

There are only a few studies on mosquito immunotherapy. The natural history of mosquito allergy suggests that after prolonged exposure to mosquito bites, desensitization may occur. This is accompanied by a decrease in the serum levels of specific IgE and an increase in specific IgG antibodies [21, 48]. The abundance of *A. communis* in Finland in the summer is accompanied by increasing levels of IgE, IgG1 and IgG4 in the population. The increase in IgG subclasses may also play a protective role [90]. These observations suggest that immunotherapy with mosquito extracts or recombinant allergens could induce a protective response against subsequent exposures. Several studies have shown that immunotherapy with allergen extracts is effective for treating mosquito allergy. In a group of 20 mosquito-allergic individuals with a positive skin test and positive specific IgE determinations to *A. communis*, the subcutaneous application of whole-body extract for 18 months induced the disappearance of the skin lesions as well as respiratory and eye symptoms that appeared after the mosquito bite. The treatment also reduced the need for antiallergic medication. These effects were not observed in the control group that did not receive immunotherapy. No adverse reactions occurred during the treatment [91]. A randomized, double-blind, placebo-controlled trial showed that immunotherapy with extracts of *C. quinquefasciatus* for 1 year in 40 patients with asthma and/or rhinitis improved allergic symptoms and decreased bronchial hyperreactivity [92]. In addition, there was a slight decrease in IgE levels accompanied by a significant increase in IgG4, which is indicative of effective immunotherapy. These changes were not observed in the placebo group [92]. Administration of mosquito extracts mixed with extracts of other insects has shown efficacy in treating allergy to various insects. In another double-blind, placebo-controlled study, the group of patients treated for 12 months with one or mixed extracts of cockroach, mos-

quito or fly, showed decreased skin test reactivity and lower IgE levels after the 12 months of immunotherapy. Moreover, skin test results, allergic symptoms and bronchial hyperreactivity were significantly lower in the group treated with the extract compared with the placebo group. The treatment induced significantly lower IgE and higher IgG4 levels than in the placebo group [93].

Conclusions

Mosquito allergy is distributed worldwide, but is especially common in tropical regions and in areas where mosquitoes are abundant. Few studies have investigated the epidemiology and immunological mechanisms implicated in mosquito allergy. Early efforts to identify mosquito allergens focused mainly on the saliva. It was assumed that the bites were the only source of exposure to their allergens. However, airborne mosquito allergens may also play a sensitizing role. Studies on the cross-reactivity among different species of mosquito and other sources of allergens are scarce. However, the few available studies suggest an important degree of cross-reactivity between mosquitoes and other arthropods. A small number of mosquito allergens have been obtained and characterized by molecular cloning. Two recombinant allergens from *A. aegypti*, Aed a 1 and Aed a 2, have been isolated and obtained as highly purified molecules. The rate of sensitization to these two allergens is often less than 50%, which suggests that other allergens may play an important role. Studies have shown that allergen immunotherapy with mosquito-allergenic extracts is effective. More research is needed to produce a more specific diagnosis and effective treatment for mosquito allergies.

Acknowledgements

José Fernando Cantillo is the recipient of a fellowship from Colciencias, Colombia, at the Universidad Complutense de Madrid, Spain. This work was supported in part by the University Corporation Rafael Nuñez, Cartagena, Colombia. The authors would like to thank Dr. Sylvie Lafosse-Marin for her collaboration in the research projects.

Disclosure Statement

The authors report no conflict of interests in relation to this paper.

References

- 1 Jaeger E: A Source-Book of Biological Names and Terms. Springfield, Thomas, 1959.
- 2 Brown L: The New Shorter Oxford English Dictionary on Historical Principles. Oxford, Clarendon, 1993.
- 3 Ribeiro JM: Blood-feeding arthropods: live syringes or invertebrate pharmacologists? *Infect Agents Dis* 1995;4:143–152.
- 4 Harbach R: The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* 2007;1668:47.
- 5 Christophers SR: *Aedes aegypti* L. The Yellow Fever Mosquito: Its Life History, Bionomics and Structure. London, Cambridge University Press, 1960, p 77.
- 6 Mackenzie JS, Gubler DJ, Petersen LR: Emerging flaviviruses: the spread and resurgence of Japanese encephalitis, West Nile and dengue viruses. *Nat Med* 2004;10:S98–S109.
- 7 Knight KL, Stone A: A Catalog of the Mosquitoes of the World, Catalog of the Family Culicidae, ed 2. Washington, Thomas Say Found, 1977.
- 8 Hudson A, Bowman L, Orr CW: Effects of absence of saliva on blood feeding by mosquitoes. *Science* 1960;131:1730–1731.
- 9 Sun D, McNicol A, James AA, Peng Z: Expression of functional recombinant mosquito salivary apyrase: a potential therapeutic platelet aggregation inhibitor. *Platelets* 2006;17:178–184.
- 10 James AA, Blackmer K, Racioppi JV: A salivary gland-specific, maltase-like gene of the vector mosquito, *Aedes aegypti*. *Gene* 1989;75:73–83.
- 11 Grossman GL, James AA: The salivary glands of the vector mosquito, *Aedes aegypti*, express a novel member of the amylase gene family. *Insect Mol Biol* 1993;1:223–232.
- 12 Stark KR, James AA: Isolation and characterization of the gene encoding a novel factor X-directed anticoagulant from the yellow fever mosquito, *Aedes aegypti*. *J Biol Chem* 1998;273:20802–20809.
- 13 Beerntsen BT, Champagne DE, Coleman JL, Campos YA, James AA: Characterization of the sialokinase I gene encoding the salivary vasodilator of the yellow fever mosquito, *Aedes aegypti*. *Insect Mol Biol* 1999;8:459–467.
- 14 Peng Z, Beckett AN, Engler RJ, Hoffman DR, Ott NL, Simons FE: Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites. *J Allergy Clin Immunol* 2004;114:1189–1194.
- 15 Peng Z, Xu W, James AA, Lam H, Sun D, Cheng L, Simons FE: Expression, purification, characterization and clinical relevance of rAed a 1—a 68-kDa recombinant mosquito *Aedes aegypti* salivary allergen. *Int Immunol* 2001;13:1445–1452.
- 16 Peng Z, Xu W, Lam H, Cheng L, James AA, Simons FE: A new recombinant mosquito salivary allergen, rAed a 2: allergenicity, clinical relevance, and cross-reactivity. *Allergy* 2006;61:485–490.
- 17 Jeon SH, Park JW, Lee BH: Characterization of human IgE and mouse IgG1 responses to allergens in three mosquito species by immunoblotting and ELISA. *Int Arch Allergy Immunol* 2001;126:206–212.
- 18 Kausar MA, Vijayan VK, Bansal SK, Menon BK, Vermani M, Agarwal MK: Mosquitoes as sources of inhalant allergens: clinicoimmunologic and biochemical studies. *J Allergy Clin Immunol* 2007;120:3.
- 19 Wongkamchai S, Khongtak P, Leeming-sawat S, Komalamisra N, Junsong N, Kulthanan K, Wisuthsarewong W, Boitano JJ: Comparative identification of protein profiles and major allergens of saliva, salivary gland and whole body extracts of mosquito species in Thailand. *Asian Pac J Allergy Immunol* 2010;28:162–169.
- 20 Simons FE, Peng Z: Skeeter syndrome. *J Allergy Clin Immunol* 1999;104:705–707.
- 21 Kulthanan K, Wongkamchai S, Triwongwanat D: Mosquito allergy: clinical features and natural course. *J Dermatol* 2010;37:1025–1031.
- 22 Brummer-Korvenkontio H, Lappalainen P, Reunala T, Palosuo T: Detection of mosquito saliva-specific IgE and IgG4 antibodies by immunoblotting. *J Allergy Clin Immunol* 1994;93:551–555.
- 23 Engler RJ: Mosquito bite pathogenesis in necrotic skin reactors. *Curr Opin Allergy Clin Immunol* 2001;1:349–352.
- 24 Bermanian MH, Korkinejad NA, Shirkhoda S, Nabavi M, Pourpak Z: Assessment of sensitization to insect aeroallergens among patients with allergic rhinitis in Yazd City, Iran. *J Allergy Asthma Immunol* 2012;11:6.
- 25 Agarwal MK, Chaudhry S, Jhamb S, Gaur SN, Chauhan UP, Agarwal HC: Etiologic significance of mosquito (*Anopheles stephensi*) in respiratory allergy in India. *Ann Allergy* 1991;67:598–602.
- 26 Gonzalez Diaz SN, Cruz AA, Sedo Mejia GA, Rojas Lozano AA, Valenzuela EA, Vidaurre Ojeda AC: Prevalence of reactions secondary to the mosquito bites of *Aedes aegypti* at The Regional Center of Allergy and Clinical Immunology, University Hospital, de Monterrey, Nuevo Leon. *Rev Alerg Mex* 2010;57:37–43.
- 27 Penneys NS, Nayar JK, Bernstein H, Knight JW: Chronic pruritic eruption in patients with acquired immunodeficiency syndrome associated with increased antibody titers to mosquito salivary gland antigens. *J Am Acad Dermatol* 1989;21:421–425.
- 28 Smith KJ, Skelton HG 3rd, Vogel P, Yeager J, Baxter D, Wagner KF: Exaggerated insect bite reactions in patients positive for HIV. Military Medical Consortium for the Advancement of Retroviral Research. *J Am Acad Dermatol* 1993;29:269–272.
- 29 Kimura H, Ito Y, Kawabe S, Gotoh K, Takahashi Y, Kojima S, Naoe T, Esaki S, Kikuta A, Sawada A, et al: EBV-associated T/NK-cell lymphoproliferative diseases in nonimmunocompromised hosts: prospective analysis of 108 cases. *Blood* 2012;119:673–686.
- 30 Kanno H, Onodera H, Endo M, Maeda F, Chida S, Akasaka T, Sawai T: Vascular lesion in a patient of chronic active Epstein-Barr virus infection with hypersensitivity to mosquito bites: vasculitis induced by mosquito bite with the infiltration of nonneoplastic Epstein-Barr virus-positive cells and subsequent development of natural killer/T-cell lymphoma with angiodestruction. *Hum Pathol* 2005;36:212–218.
- 31 Roh EJ, Chung EH, Chang YP, Myoung NH, Jee YK, Seo M, Kang JH: A case of hypersensitivity to mosquito bites associated with Epstein-Barr viral infection and natural killer cell lymphocytosis. *J Korean Med Sci* 2010;25:321–323.
- 32 Diven DG, Newton RC, Ramsey KM: Heightened cutaneous reactions to mosquito bites in patients with acquired immunodeficiency syndrome receiving zidovudine. *Arch Intern Med* 1988;148:2296.
- 33 Asada H, Saito-Katsuragi M, Niizeki H, Yoshioka A, Suguri S, Isonokami M, Aoki T, Ishihara S, Tokura Y, Iwatsuki K, Miyagawa S: Mosquito salivary gland extracts induce EBV-infected NK cell oncogenesis via CD4 T cells in patients with hypersensitivity to mosquito bites. *J Invest Dermatol* 2005;125:956–961.
- 34 Nyakeriga MA, Troye-Blomberg M, Bereczky S, Perlmann H, Perlmann P, ElGhazali G: Immunoglobulin E (IgE) containing complexes induce IL-4 production in human basophils: effect on Th1-Th2 balance in malaria. *Acta Trop* 2003;86:55–62.
- 35 Mecheri S: Contribution of allergic inflammatory response to the pathogenesis of malaria disease. *Biochim Biophys Acta* 2012;1822:49–56.
- 36 Valença LM, Restivo PC, Nunes MS: Seasonal variations in emergency room visits for asthma attacks in Gama, Brazil. *J Bras Pneumol* 2006;32:284–289.
- 37 Fernandez-Caldas E, Puerta L, Mercado D, Lockett RF, Caraballo LR: Mite fauna, Der p I, Der f I and *Blomia tropicalis* allergen levels in a tropical environment. *Clin Exp Allergy* 1993;23:292–297.
- 38 Puerta L, Fernandez-Caldas E, Lockett RF, Caraballo LR: Mite allergy in the tropics: sensitization to six domestic mite species in Cartagena, Colombia. *J Invest Allergol Clin Immunol* 1993;3:198–204.
- 39 Montoya-Lerma J, Solarte YA, Giraldo-Calderon GI, Quinones ML, Ruiz-Lopez F, Wilkerson RC, Gonzalez R: Malaria vector species in Colombia: a review. *Mem Inst Oswaldo Cruz* 2011;106(suppl 1):223–238.
- 40 Morrone JJ: Biogeographic areas and transition zones of Latin America and the Caribbean islands based on panbiogeographic and cladistic analyses of the entomofauna. *Annu Rev Entomol* 2006;51:467–494.

- 41 White GB: Medical acarology and entomology: mosquitoes; in Cook GC, Zumla AI (eds): *Manson's Tropical Diseases*, ed 21. London, Elsevier, 2004.
- ▶ 42 Mercado D, Puerta L, Caraballo L: Life-cycle of *Suidasia medanensis* (= *pontifica*) (Acari: Suidasiidae) under laboratory conditions in a tropical environment. *Exp Appl Acarol* 2001; 25:751–755.
- ▶ 43 Peng Z, Simons FE: Cross-reactivity of skin and serum-specific IgE responses and allergen analysis for three mosquito species with worldwide distribution. *J Allergy Clin Immunol* 1997;100:192–198.
- ▶ 44 Peng Z, Li H, Simons FE: Immunoblot analysis of salivary allergens in 10 mosquito species with worldwide distribution and the human IgE responses to these allergens. *J Allergy Clin Immunol* 1998;101:498–505.
- ▶ 45 Cabrerizo Ballesteros S, de Barrio M, Baeza ML, Rubio Sotes M: Allergy to chironomid larvae (red midge larvae) in nonprofessional handlers of fish food. *J Investig Allergol Clin Immunol* 2006;16:63–68.
- ▶ 46 Hallas TE, Gislason D, Bjornsdottir US, Jorundsdottir KB, Janson C, Luczynska CM, Gislason T: Sensitization to house dust mites in Reykjavik, Iceland, in the absence of domestic exposure to mites. *Allergy* 2004;59:515–519.
- ▶ 47 Mackay I, Rose N: Allergic diseases; in Mackay I, Rose NR, Ledford DK, Lockey RF (eds): *Encyclopedia of Medical Immunology*. New York, Springer, 2014.
- ▶ 48 Peng Z, Simons FE: A prospective study of naturally acquired sensitization and subsequent desensitization to mosquito bites and concurrent antibody responses. *J Allergy Clin Immunol* 1998;101:284–286.
- ▶ 49 Peng Z, Yang M, Simons FE: Immunologic mechanisms in mosquito allergy: correlation of skin reactions with specific IgE and IgG antibodies and lymphocyte proliferation response to mosquito antigens. *Ann Allergy Asthma Immunol* 1996;77:238–244.
- ▶ 50 Walker GB, Harrison PV: Seasonal bullous eruption due to mosquitoes. *Clin Exp Dermatol* 1985;10:127–132.
- ▶ 51 Mellanby K: Man's reaction to mosquito bites. *Nature* 1946;158:554.
- ▶ 52 Heilesen B: Studies on mosquito bites. *Acta Allergol* 1949;2:245–267.
- ▶ 53 McKiel JA, West AS: Effects of repeated exposures of hypersensitive humans and laboratory rabbits to mosquito antigens. *Can J Zool* 1961;39:7.
- ▶ 54 Wynn SR, Swanson MC, Reed CE, Penny ND, Showers WB, Smith JM: Immunochemical quantitation, size distribution, and cross-reactivity of Lepidoptera (moth) aeroallergens in southeastern Minnesota. *J Allergy Clin Immunol* 1988;82:47–54.
- ▶ 55 Ostrom NK, Swanson MC, Agarwal MK, Yunginger JW: Occupational allergy to honeybee-body dust in a honey-processing plant. *J Allergy Clin Immunol* 1986;77:736–740.
- ▶ 56 Swanson MC, Agarwal MK, Reed CE: An immunochemical approach to indoor aeroallergen quantitation with a new volumetric air sampler: studies with mite, roach, cat, mouse, and guinea pig antigens. *J Allergy Clin Immunol* 1985;76:724–729.
- ▶ 57 Peng Z, Ho MK, Li C, Simons FE: Evidence for natural desensitization to mosquito salivary allergens: mosquito saliva specific IgE and IgG levels in children. *Ann Allergy Asthma Immunol* 2004;93:553–556.
- ▶ 58 Reunala T, Brummer-Korvenkontio H, Rasanen L, Francois G, Palosuo T: Passive transfer of cutaneous mosquito-bite hypersensitivity by IgE anti-saliva antibodies. *J Allergy Clin Immunol* 1994;94:902–906.
- ▶ 59 Reunala T, Brummer-Korvenkontio H, Palosuo K, Miyanij M, Ruiz-Maldonado R, Love A, Francois G, Palosuo T: Frequent occurrence of IgE and IgG4 antibodies against saliva of *Aedes communis* and *Aedes aegypti* mosquitoes in children. *Int Arch Allergy Immunol* 1994;104:366–371.
- ▶ 60 Horsmanheimo L, Harvima IT, Harvima RJ, Brummer-Korvenkontio H, Francois G, Reunala T: Histamine and leukotriene C4 release in cutaneous mosquito-bite reactions. *J Allergy Clin Immunol* 1996;98:408–411.
- ▶ 61 Chen YL, Simons FE, Peng Z: A mouse model of mosquito allergy for study of antigen-specific IgE and IgG subclass responses, lymphocyte proliferation, and IL-4 and IFN-gamma production. *Int Arch Allergy Immunol* 1998;116:269–277.
- ▶ 62 Demeure CE, Brahimi K, Hacini F, Marchand F, Peronet R, Huerre M, St-Mezard P, Nicolas JF, Brey P, Delespesse G, Mecheri S: Anophelines mosquito bites activate cutaneous mast cells leading to a local inflammatory response and lymph node hyperplasia. *J Immunol* 2005;174:3932–3940.
- ▶ 63 Abdel-Naser MB, Lotfy RA, Al-Sherbiny MM, Sayed Ali NM: Patients with papular urticaria have IgG antibodies to bedbug (*Cimex lectularius*) antigens. *Parasitol Res* 2006;98:550–556.
- ▶ 64 Peng Z, Simons FE: Mosquito allergy: immune mechanisms and recombinant salivary allergens. *Int Arch Allergy Immunol* 2004;133:198–209.
- ▶ 65 Heng MC, Kloss SG, Habermelde GC: Pathogenesis of papular urticaria. *J Am Acad Dermatol* 1984;10:1030–1034.
- ▶ 66 Alexander JO: Papular urticaria and immune complexes. *J Am Acad Dermatol* 1985;12:374–375.
- ▶ 67 Wongkamchai S, Techasintana P, Wisuthsarewong W, Kulthanan K, Suthipinittharm P, Eakpo P: Analysis of IgE-binding allergens in *Culex quinquefasciatus* saliva protein in mosquito bite-allergic patients. *Ann Allergy Asthma Immunol* 2007;98:200–201.
- ▶ 68 Peng Z, Simons FE: Comparison of proteins, IgE, and IgG binding antigens, and skin reactivity in commercial and laboratory-made mosquito extracts. *Ann Allergy Asthma Immunol* 1996;77:371–376.
- ▶ 69 Agarwal MK, Yunginger JW, Swanson MC, Reed CE: An immunochemical method to measure atmospheric allergens. *J Allergy Clin Immunol* 1981;68:194–200.
- ▶ 70 Trudeau WL, Fernandez-Caldas E, Fox RW, Brenner R, Bucholtz GA, Lockey RF: Allergenicity of the cat flea (*Ctenocephalides felis felis*). *Clin Exp Allergy* 1993;23:377–383.
- ▶ 71 Cantillo JF, Puerta L, Fernandez-Caldas E, Lafosse-Marin S: Characterization of the allergenic activity of tropomyosin from *Aedes aegypti*. *J Allergy Clin Immunol* 2014;133:1.
- ▶ 72 Ribeiro JM, Sarkis JJ, Rossignol PA, Spielman A: Salivary apyrase of *Aedes aegypti*: characterization and secretory fate. *Comp Biochem Physiol B* 1984;79:81–86.
- ▶ 73 Champagne DE, Smartt CT, Ribeiro JM, James AA: The salivary gland-specific apyrase of the mosquito *Aedes aegypti* is a member of the 5'-nucleotidase family. *Proc Natl Acad Sci U S A* 1995;92:694–698.
- ▶ 74 Xu W, Simons FE, Peng Z: Expression and rapid purification of an *Aedes aegypti* salivary allergen by a baculovirus system. *Int Arch Allergy Immunol* 1998;115:245–251.
- ▶ 75 Reno HE, Novak RJ: Characterization of apyrase-like activity in *Ochlerotatus triseriatus*, *Ochlerotatus hendersoni*, and *Aedes aegypti*. *Am J Trop Med Hyg* 2005;73:541–545.
- ▶ 76 Calvo E, Mans BJ, Ribeiro JM, Andersen JF: Multifunctionality and mechanism of ligand binding in a mosquito anti-inflammatory protein. *Proc Natl Acad Sci U S A* 2009;106:3728–3733.
- ▶ 77 James AA, Blackmer K, Marinotti O, Ghosn CR, Racioppi JV: Isolation and characterization of the gene expressing the major salivary gland protein of the female mosquito, *Aedes aegypti*. *Mol Biochem Parasitol* 1991;44:245–253.
- ▶ 78 Peng Z, Lam H, Xu W, Cheng L, Chen YL, FER S: Characterization and clinical relevance of two recombinant mosquito *Aedes aegypti* salivary allergens, rAed a 1 and rAed a 2. *J Allergy Clin Immunol* 1998;101:32.
- ▶ 79 Nascimento EP, dos Santos Malafronte R, Marinotti O: Salivary gland proteins of the mosquito *Culex quinquefasciatus*. *Arch Insect Biochem Physiol* 2000;43:9–15.
- ▶ 80 Malafronte Rdos S, Calvo E, James AA, Marinotti O: The major salivary gland antigens of *Culex quinquefasciatus* are D7-related proteins. *Insect Biochem Mol Biol* 2003;33:63–71.
- ▶ 81 Xu W, Peng Z, Simons FER: Isolation of a cDNA encoding a 30-kDa IgE-binding protein of mosquito *Aedes aegypti* saliva. *J Allergy Clin Immunol* 1998;101:203.
- ▶ 82 Beckett AN, Sun W, Simons FER, Ma Y, Peng Z: Role of recombinant mosquito salivary allergens in the diagnosis of individuals with allergic reactions to mosquito bites. *J Allergy Clin Immunol* 2004;13:74.
- ▶ 83 Li C, Beckett AN, Simons FER, Li C, Zhang T, Peng Z: A new 67-kDa recombinant *Aedes aegypti* salivary allergen rAed a 4 in the diagnosis of mosquito allergy. *J Allergy Clin Immunol* 2005;115(suppl 2):S100.

- ▶84 Puerta L, Lagares A, Mercado D, Fernandez-Caldas E, Caraballo L: Allergenic composition of the mite *Suidasia medanensis* and cross-reactivity with *Blomia tropicalis*. *Allergy* 2005;60:41–47.
- 85 Fernández-Caldas E, Puerta L, Caraballo L, Lockey RF: Mite allergens; in Locke R, Ledford D (eds): *Allergens and Allergen Immunotherapy: Subcutaneous, Sublingual, and Oral*, ed 5. London, Informa Healthcare, 2014.
- ▶86 Sabbah A, Hassoun S, Drouet M, Lauret MG, Doucet M: The wasp/mosquito syndrome. *Allerg Immunol (Paris)* 1999;31:175–184.
- ▶87 Adalsteinsdottir B, Sigurdardottir S, Gislason T, Kristensen T, Gislason D: What characterizes house dust mite-sensitive individuals in a house dust mite free community in Reykjavik, Iceland? *Allergol Int* 2007;56:6.
- ▶88 Valenta R, Lidholm J, Niederberger V, Hayek B, Kraft D, Gronlund H: The recombinant allergen-based concept of component-resolved diagnostics and immunotherapy (CRD and CRIT). *Clin Exp Allergy* 1999;29:896–904.
- 89 Cantillo JF, Lafosse-Marin S, Fernandez-Caldas E, Puerta L: Mosquito allergy in the tropics: involvement of tropomyosin and cross-reactivity with other arthropods. *Allergy* 2014;69:432.
- ▶90 Palosuo K, Brummer-Korvenkontio H, Mikkola J, Sahi T, Reunala T: Seasonal increase in human IgE and IgG4 antisaliva antibodies to *Aedes* mosquito bites. *Int Arch Allergy Immunol* 1997;114:367–372.
- ▶91 Ariano R, Panzani RC: Efficacy and safety of specific immunotherapy to mosquito bites. *Eur Ann Allergy Clin Immunol* 2004;36:131–138.
- ▶92 Srivastava D, Singh BP, Sudha VT, Arora N, Gaur SN: Immunotherapy with mosquito (*Culex quinquefasciatus*) extract: a double-blind, placebo-controlled study. *Ann Allergy Asthma Immunol* 2007;99:273–280.
- ▶93 Srivastava D, Singh BP, Arora N, Gaur SN: Clinico-immunologic study on immunotherapy with mixed and single insect allergens. *J Clin Immunol* 2009;29:665–673.