

Advances in mosquito allergy

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Purpose of review

Allergic reactions, including severe local and systemic reactions to mosquito bites, are immunological in nature, and involve immunoglobulin E, immunoglobulin G, and T-lymphocyte-mediated hypersensitivities in response to allergens in mosquito saliva. Naturally acquired desensitization to mosquito saliva may occur during childhood or during long-term exposure to mosquitoes. Due to the lack of availability of mosquito salivary preparations for use in skin tests and in-vitro tests, allergic reactions to mosquito bites are under diagnosed and under treated.

Recent findings

Recombinant saliva allergens with biological activity are being developed. Recombinant *Aedes aegypti* salivary allergen rAed a 2 has been expressed, purified, characterized and used in in-vitro diagnosis of mosquito allergy. Mosquito saliva-induced non-immunoglobulin E-mediated skin mast cell degranulation was found to induce macrophage-inflammatory protein 2 in the skin and interleukin-10 in draining lymph nodes.

Summary

In this review, we discuss the allergic reactions to mosquito salivary allergens, the immune mechanisms involved, natural desensitization and immunotherapy with mosquito extracts, characteristics of salivary allergens and their recombinant forms, and prevention and treatment of allergic reactions to mosquito bites. Eventually, recombinant salivary allergens will significantly improve the diagnosis of mosquito allergy, and will also improve specific immunotherapy for patients with systemic reactions to mosquito bites.

Keywords

immunoglobulin E, mosquito allergy, recombinant antigen, salivary allergen

Introduction

Allergic reactions to mosquito bites are common, and may decrease quality of life [1]. Severe local reactions such as 'Skeeter syndrome' [2], and systemic reactions including generalized urticaria and anaphylaxis, have been reported [3]. Due to the lack of availability of mosquito salivary preparations, which are required for accurate diagnosis and immunotherapy, these reactions are under recognized and allergen immunotherapy is seldom used for severe reactions to mosquito bites. For the same reason, the immune mechanisms associated with the development of allergic reactions and subsequent natural desensitization after exposure to mosquito bites are not yet completely understood. Utilization of molecular biology techniques to produce pure mosquito salivary allergens has made it possible to investigate the immune responses involved and to diagnose mosquito allergy accurately, and eventually will make it possible to treat people who have severe allergic reactions to mosquito bites with standardized high-quality, specific allergen immunotherapy.

Clinical manifestations

Typical local cutaneous reactions to mosquito bites consist of immediate wheals and flares peaking at 20 min and delayed pruritic indurated papules peaking at 24–36 h, then diminishing over several days or weeks [1]. Large local reactions to mosquito bites consist of itchy, red, warm swellings appearing within minutes of the bites, and itchy papules, ecchymotic, vesiculated, blistering, bullous reactions or even Arthus reactions, appearing 2–6 h after the bites and persisting for days or weeks. Only those reactions which cause large or atypical (ecchymotic or vesiculated) local reactions or systemic reactions should be described as 'mosquito allergy' [4]. 'Skeeter syndrome' is defined as a mosquito saliva-induced large local inflammatory reaction sometimes accompanied by low-grade fever that is often misdiagnosed as cellulitis. Systemic reactions following mosquito bites include angioedema, generalized urticaria, nausea, vomiting, wheezing and other anaphylaxis manifestations [3,5].

Individuals at increased risk of severe reactions to mosquito bites include those with a high level of exposure, for example, outdoor workers [5]; those with weak natural immunity, for example, infants and young children [2,6]; those who have had no previous exposure to indigenous mosquitoes, for example, immigrants or visitors; and individuals with Epstein-Barr virus-associated natural killer/T lymphoproliferative diseases [7–13], who may

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Abbreviations

ELISA enzyme-linked immunosorbent assay
Ig immunoglobulin

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develop necrotic skin ulcers at the sites of mosquito bites. Epstein–Barr virus-infected natural killer/T cells play an important role in the pathogenesis of mosquito allergy. Cytotoxic chemotherapy successfully improves the symptoms of mosquito allergy in these patients [10].

Immune mechanisms

Reactions to mosquito bites are immunological in nature. They are due to specific sensitization to the mosquito salivary proteins, because initial exposure to mosquito species to which an individual has not been previously exposed causes no reaction. Mosquito saliva-specific immunoglobulin (Ig)-E and IgG antibodies as well as lymphocyte proliferation appear to be involved in the pathogenesis of allergic reactions to mosquito bites [1]. In local allergic reactions to mosquito bites, mosquito-saliva-specific IgE and IgG levels and the mosquito allergen-induced lymphocytes proliferation index are elevated. Both IgE and IgG levels correlate with mosquito bite-induced skin immediate and delayed reactions, while lymphocyte responses to mosquito allergens correlate with skin delayed reactions [1]. In systemic reactions, however, only mosquito saliva-specific IgE is involved [3].

Non-IgE-mediated mast cell degranulation may also be involved in the mechanism of mosquito-bite induced allergic responses. Recently, mosquito bite-induced skin mast cell degranulation leading to fluid extravasation and recruitment of neutrophils at the site of the mosquito bite was reported in a murine model. *Anopheles stephensi* saliva directly induces isolated connective tissue-type but not mucosal-type mast cells to degranulate *in vitro* in the absence of IgE antibodies. This inflammatory response did not occur in mast cell-deficient mice, unless these mice were reconstituted specifically with mast cells [14]. Further, it was reported that mosquito bite-induced skin mast cell degranulation induces macrophage-inflammatory protein 2 (MIP-2) in the skin and interleukin (IL)-10 in draining lymph nodes, which downregulated other antigen-specific T-cell responses [15].

Natural desensitization and immunotherapy

Based on clinical and experimental observations, the natural history of sensitization and subsequent desensitization to the saliva injected by mosquito bites has been documented [1]. In a prospective human study, the entire sensitization and subsequent desensitization process took approximately 26 weeks [16]. In real life, natural desensitization may take years to occur or may never occur, since mosquito-allergic individuals generally try to avoid getting bitten by mosquitoes. Naturally acquired desensitization to mosquito saliva has also been confirmed in several cross-sectional studies [1]. In a study of 401 infants, children, and adolescents ranging in age from 1 month to 18 years, both mosquito saliva specific IgE and

IgG antibodies gradually declined with age, and there was a significant inverse correlation between age and IgE and IgG levels [6]. In adolescents aged 16–18 years, the mean levels of saliva-specific IgE and IgG antibodies were similar to those reported in 1059 adults by the same authors [17], using the same immunoassay. The results from these two studies suggest that natural desensitization probably occurs during childhood and adolescence, and that infants and young children may be at increased risk of having severe reactions to mosquito bites.

Based on the above observations, immunotherapy with injections of gradually increasing doses of mosquito saliva proteins may be expected to prevent reactions to mosquito bites, and immunotherapy using injections of mosquito whole body extracts has confirmed this [1,18]. Ariana and Panzani [18] treated 20 mosquito-allergic individuals with *Aedes communis* whole body extract for 18 months. Treated individuals reported the disappearance of severe local reactions and symptoms of allergic rhinitis which was statistically correlated with improvement of symptom and medication consumption scores and decreased allergic reactivity in nasal provocation tests with mosquito extract. This therapy, however, is neither well studied nor widely used in mosquito allergy, because currently commercially available mosquito whole body extracts contain few mosquito saliva proteins and many nonsalivary proteins which are ineffective in downregulating the specific immune responses to mosquito salivary allergens and may even cause additional sensitization [19].

Mosquito salivary allergens

It is the proteins in mosquito saliva to which people develop either natural desensitization, large local reactions or systemic allergic reactions. Mosquito saliva contains many biological materials [1], for example, *Aedes aegypti* saliva contains at least 31 proteins, for which the cDNA sequences have been deposited into the Genbank [20]. Most sequences match known proteins, although some do not, and most of the proteins in mosquito saliva are allergenic in humans [1,20,21]. Table 1 summarizes the characteristics of major salivary proteins in *A. aegypti*. Using immunoblot analyses with a pooled serum from individuals allergic to mosquito bites, allergens in the saliva or salivary gland extracts from 10 worldwide distributed mosquito species have been studied and three to 16 salivary allergens are found in each species, with molecular masses ranging from 16 to 95 kDa. Both species-shared and species-specific allergens exist [22]. IgE antibodies against 35.5, 32.5, and 22.5 kDa allergens in *Culex quinquefasciatus* saliva were found in 22 (41%), 15(28%), and 13(24%) of 54 individuals with a history of mosquito bite allergy, respectively. The 22.5 kDa allergen was found mainly in children [23]. In another study, serum saliva-specific IgE levels to five

Table 1 *Aedes aegypti* salivary proteins

Protein name	Allergen name	Molecular weight (kDa)	cDNA sequenced	Biological functions
α -Amylase 1		81.5	Yes	Unknown
apyrase	Aed a 1	68	Yes	Antiplatelet
α -Glucosidase (maltase-like 1)	Aed a 4	67	Yes	Sugar digestion
Esterase		65		Unknown
Anticoagulant-factor Xa		54	Yes	Anticoagulant
Aed a X ₁	Aed a X ₁ ?	44		Unknown
Aed a X ₂	Aed a X ₂ ?	37		Unknown
Female-specific protein, D7	Aed a 2	37	Yes	Unknown
	Aed a 3	30	Yes	Unknown
Sialokinins		1.4	Yes	Vasodilator
Antitumour necrosis factor		Unknown		Antitumour
Lysozyme		Unknown		Bacteriolysis

Reproduced from Peng and Simons [1].

common mosquito species were measured in 14 individuals with a history of systemic reactions to mosquito bites [3]. Eleven of these individuals had a positive IgE response to the saliva of *Aedes albopictus* [3], which contains 16 allergens [22], while five to eight individuals had a positive IgE response to each of the remaining four species, suggesting that *Ae. albopictus* is the most common species associated with systemic allergic reactions to mosquito bites [3].

Recombinant mosquito salivary allergens

Mosquito saliva can be obtained only by dissecting out the salivary glands or by direct collection of saliva from living female mosquitoes. Each salivation of *Aedes vexans* contains only 0.2 μ g of proteins [1]. Both processes are extremely tedious, and therefore not practical. Utilization of molecular engineering methods to produce pure mosquito salivary allergens on a large scale has provided an important research tool for investigation of the mechanisms involved, and will eventually facilitate diagnosis and treatment of mosquito allergy with standardized, effective, and safe allergens. *Ae. aegypti* is the major mosquito pest in the world, and shares many salivary allergens with other species [1]. To date, four recombinant salivary proteins of *Ae. aegypti*, a 68 kDa apyrase referred to as recombinant Aed a 1 (rAed a 1) [24], a 37 kDa D7 protein known as rAed a 2 [25[•]], a 30 kDa Aed a 3 [26], and a 67 kDa α -glucosidase called rAed a 4 [27], have been expressed and identified as allergens (Table 1).

These four recombinant allergens have been expressed using a baculovirus/insect cell system, which has been used extensively for the production of large amounts of recombinant proteins with biological activity [24,25[•],26,27]. They have been demonstrated to bind to serum IgE, to induce histamine release from basophils, and to induce skin immediate and delayed reactions in individuals with allergic reactions to mosquito bites [1,24,25[•],26,27]. Furthermore, the binding of IgE to the recombinant allergen in the enzyme-linked immunosorbent assay (ELISA) can be inhibited by the addition

of natural salivary preparations in a dose-dependent manner, and the sizes of recombinant mosquito salivary allergen-induced skin flare correlate significantly with those of mosquito bite-induced flares. Both native Aed a 1 and Aed a 2 in the saliva and rAed a 1 and rAed a 2 expressed reacted with the IgE in mosquito-allergic human serum and with a rabbit antibody against rAed a 1 or a rabbit antibody against rAed a 2, respectively [24,25[•]]. These recombinant allergens have identical immunogenicity to their natural forms present in the saliva. They also have similar biological activities as shown by eliciting skin reactions, histamine release, and other biological functions; for example, rAed a 1 (apyrase) inhibits ADP, collagen and thrombin-induced human platelet aggregation in a dose-dependent manner [28].

Cross reactivity exists among the salivary allergens of different mosquito species [1]. This enhances diagnostic sensitivity in mosquito allergy and suggests that immunotherapy in patients with systemic reactions to mosquito bites using the salivary allergen from one species may protect them against reactions triggered by another mosquito species. In immunoblot analysis with a rabbit antiAed a 1 antibody, a 68 kDa protein was found in three of the four species studied [24], while using a rabbit antiAed a 2, a 37 kDa protein was found in all five *Aedes* species studied, *Ochlerotatus triseriatus* and *Culex quinquefasciatus*, but it was not present in three other species [25[•]].

Diagnosis

A history of an allergic reaction which develops following a witnessed mosquito bite is helpful in diagnosing mosquito allergy; however, most bites are painless and not directly observed, especially in young children. Immunoassays have been developed to measure mosquito salivary specific IgE antibodies. The UniCap test, in which a mosquito whole body extract is used as the capture antigen, was compared with a mosquito saliva-capture ELISA in the diagnosis of individuals with

systemic or severe local reactions to mosquito bites [29]. The mosquito saliva-capture ELISA was more sensitive and specific (less likely to give false negative and false positive results) than the UniCap test in the diagnosis of mosquito allergy. The positivity rate was 56% for the saliva-capture ELISA and 25% for the UniCap test in mosquito-allergic individuals, versus 5% in the negative controls, suggesting that only mosquito saliva proteins should be used in the diagnosis of mosquito allergy.

To investigate the clinical relevance of the recombinant allergens, skin prick tests with rAed a 1, rAed a 2, and rAed a 3, as well as mosquito bite tests, were performed in healthy volunteers with skin reactivity ranging from none to strong local reactions to mosquito bites [24,25^{*},30,31]. In all mosquito bite tests, laboratory-reared, pathogen-free mosquitoes were used. Using the mean of the mosquito-bite test negative individuals plus 2SD as a cutoff level, 29–43% of mosquito bite test-positive individuals had a positive skin test to rAed a 1, 10% to rAed a 2, and 32% to rAed a 3, and none of the individuals with negative bite tests had a positive skin test to any of these three recombinant allergens [24,25^{*},30,31].

Moreover, sensitive and specific indirect ELISAs using the recombinant allergens are being developed to measure serum allergen-specific IgE levels. In the ELISAs, the recombinant salivary allergens are coated on microplates. In one study [27], 47% of the mosquito-allergic individuals had a positive rAed a 4-specific IgE level, and none of the negative controls had a positive IgE, while in another study [32], the positivity rates of specific IgE were 65% for rAed a 1, 32% for rAed a 2 and rAed a 3, and 81% for saliva in the allergic group, and 0–10% in the negative controls ($P < 0.05$). Levels of specific IgE and IgG to each recombinant allergen significantly correlated with those of saliva-specific antibodies. The positivity rates of rAed a 1, rAed a 3, and saliva-specific IgG were also significantly increased in the allergic individuals in comparison with the controls ($P < 0.05$) [32]. These results indicate that sensitive and specific in-vitro immunoassays can be developed by using recombinant mosquito salivary allergens.

Prevention of mosquito bites

Individuals with mosquito allergy should avoid mosquito-infested areas such as tall grass, bush, wetlands and swamps. They should wear protective clothing when exposure to mosquitoes is anticipated. Personal mosquito repellents such as *N,N*-diethyl-*m*-toluamide (DEET) should be applied to exposed skin. DEET in a concentration of 10% can be safely applied to the skin of infants over the age of 6 months, and to young children. Clothing, bed nets and camping tents should be treated with an insecticide such as permethrin [4].

Treatment of local and systemic allergic reactions to mosquito bites

In laboratory and field studies, prophylactic administration of a second-generation H_1 -antihistamine such as cetirizine or levocetirizine is effective in reducing itching in the early-phase allergic response, and contributes to downregulation of the late-phase response [4,33]. For mild late-phase reactions that have already occurred, a glucocorticoid cream such as mometasone furoate 1 mg/g should be applied to the site of the reaction twice daily for 5–7 days; however, for severe large local reactions, oral administration of prednisone 1 mg/kg daily (maximum 50 mg) for 5–7 days will be needed. Antibiotic treatment is not indicated for large local reactions that have developed within 24 h of a mosquito bite. In rare cases, individuals who are at risk for anaphylaxis (a serious allergic reaction that is rapid in onset and may cause death) from mosquito bites should carry self-injectable epinephrine whenever they are likely to be exposed to mosquitoes [4].

Conclusion

Most people who are bitten by mosquitoes get sensitized to mosquito saliva, and some of them develop mosquito allergy which includes large local and, rarely, systemic reactions. Due to the lack of salivary preparations, allergic reactions to mosquito bites are under diagnosed and under treated. Recombinant mosquito salivary allergens with biological activity are now available in a few centres, and the diagnosis of mosquito allergy is moving from a descriptive, empirical basis to an objective, scientific basis using recombinant salivary allergens. A mixture of these recombinant allergens will improve diagnosis of mosquito allergy and eventually will also improve specific immunotherapy for individuals with severe local and systemic reactions to mosquito bites.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 361).

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