

Mosquito Allergy: Recombinant Mosquito Salivary Antigens for New Diagnostic Tests

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Key Words

Mosquito allergy · Recombinant antigen · Local cutaneous allergic response · Anaphylaxis · Skin test · ELISA

Abstract

In patients with mosquito allergy, lack of a readily available, sensitive, specific, safe test is the major obstacle to accurate diagnosis. Three recombinant mosquito salivary antigens, rAed a 1 (68 kD), rAed a 2 (37 kD) and rAed a 3 (30 kD), from *Aedes aegypti* have been cloned, expressed, purified and characterized. All three recombinant antigens are shared by *Aedes vexans* and other mosquito species, and all have been found to have biologic activity in humans. In recent studies, 43% of 28 mosquito bite test-positive subjects had a positive skin test to rAed a 1, 11% to rAed a 2 and 32% to rAed a 3. The sizes of the skin test reactions to the recombinant antigens correlated with the sizes of the *A. aegypti* bite test reactions. None of 15 *A. aegypti* bite test-negative subjects had a positive skin test to any of the recombinant antigens. Recombinant mosquito salivary antigens will facilitate the diagnosis of mosquito allergy.

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Introduction

Large local allergic reactions to mosquito bites are common, and the prevalence of systemic allergic reactions to mosquito bites is increasing [1–3]. These reactions are underrecognized, and are sometimes erroneously assumed to have an infectious or toxic etiology, when in fact they are caused by mosquito saliva which induces early- and/or late-phase allergic reactions involving IgE and IgG, especially IgG₄ [2, 4]. Optimal diagnosis and treatment of allergic reactions to mosquitoes are limited by the lack of availability of high-quality specific allergens.

Persons at increased risk for mosquito allergy include otherwise healthy young children, immigrants, visitors or outdoor workers with little previous exposure to mosquitoes indigenous to a particular area, and patients with primary or secondary immunodeficiency disorders.

Although patients with mosquito allergy commonly present with large local reactions, urticaria, anaphylaxis and other systemic manifestations have also been reported [2, 3]. Natural desensitization to the saliva injected in mosquito bites eventually occurs, but this is exposure-dependent and may take years [4–6].

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Diagnosis of Mosquito Allergy

A history of an allergic reaction following a witnessed mosquito bite is obviously helpful in diagnosing mosquito allergy, but as mosquito bites are painless and often unwitnessed, a definitive cause-and-effect relationship between the bite and the reaction is seldom apparent.

Lack of readily available, sensitive, specific, safe tests for mosquito allergy is the major obstacle to accurate diagnosis. Options include mosquito bite challenge tests (*not recommended*), skin tests and in vitro tests. The potential problems associated with the use of a mosquito bite challenge test for diagnostic purposes include: lack of availability of the insect, the risk of transmitting disease through the bite and the risk of causing a large local reaction or a systemic reaction, including anaphylaxis, in a susceptible patient. Mosquito bite challenge tests using laboratory-reared, disease-free mosquitoes in carefully screened subjects are, however, useful in mosquito allergy research.

Skin tests performed using commercially available, nonstandardized mosquito whole body extracts cannot be relied upon for diagnosis. These extracts often result in false-negative tests due to low content of the relevant mosquito saliva antigens, or false-positive tests due to irritating nonallergenic proteins [7].

In a few centers, in vitro tests in which mosquito salivary gland extract or saliva is used as the allergen are available for diagnostic purposes. These tests include specific, sensitive ELISAs for measurement of IgE, IgG, IgG₁ and IgG₄, as well as RASTs, immunoblotting and lymphocyte transformation tests [2, 4, 6, 8–11].

An important goal in mosquito allergy research is the development of a dependable supply of high-quality mosquito saliva antigens for specific in vivo and in vitro diagnostic tests. Pure mosquito saliva can be obtained only by dissecting the salivary glands out of the heads and thoraces of individual mosquitoes [7, 8] or by inserting the mosquito proboscises into capillary tubes, stimulating saliva secretion and collecting, lyophilizing and reconstituting the saliva before use [7]. These techniques are extremely labor-intensive and time-consuming, and the supply of mosquito saliva antigens for use in skin tests and in vitro tests is consequently limited [7].

Shared mosquito saliva antigens have been identified among many mosquito species with worldwide distribution [12, 13]. In the saliva of *Aedes aegypti*, the most common mosquito pest worldwide [13], there are at least 8 antigens, and new ones are being discovered [14]. Three *A. aegypti* salivary antigens have been cloned, expressed,

purified and characterized utilizing molecular biology techniques [15–19]. They are rAed a 1 (68 kD), rAed a 2 (37 kD) and rAed a 3 (30 kD). All three recombinant antigens are shared by *Aedes vexans* and other mosquito species [12, 13], and all have been found to have biological activity in humans. In our recent studies, 43% of 28 mosquito bite test-positive subjects had a positive epicutaneous test to rAed a 1, 11% to rAed a 2 and 32% to rAed a 3. There was a significant correlation between skin test reactivity to the recombinant antigens and the size of the reactions to *A. aegypti* bite challenge tests (e.g. wheals rAed a 3, $r = 0.43$, $p < 0.004$; flares, $r = 0.51$, $p < 0.000$). None of 15 *A. aegypti* bite challenge test-negative subjects had a positive skin test to any of the recombinant antigens. These recombinant mosquito saliva antigens with identical biological activity to that of the natural antigens will greatly facilitate the diagnosis of mosquito allergy.

Potential Role of Recombinant Mosquito Salivary Antigens in Immunotherapy

In some patients with a history of mosquito bite-induced anaphylaxis, immunotherapy with mosquito whole body extracts is useful [3], although on an empirical basis, it would be preferable to use mosquito head and thorax extracts. Immunotherapy with pure recombinant mosquito salivary antigens is not currently feasible due to the lack of availability of such preparations. Proof of concept of the usefulness of recombinant mosquito salivary antigens in immunotherapy is currently being established in a mouse model of mosquito allergy [20–22].

Summary

Mosquito allergy is underrecognized and undertreated. As high-quality mosquito saliva antigens have become available, the diagnosis of mosquito allergy has moved from a descriptive and anecdotal basis to an objective and scientific basis. Recombinant mosquito saliva antigens will be useful for the diagnosis of mosquito allergy, and eventually will lead to improved specific immunotherapy for patients with anaphylaxis to mosquitoes.

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