

Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites

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Background: Mosquito bite-induced acute systemic allergic reactions are an increasing clinical concern and have not been optimally characterized immunologically.

Objective: We wanted to study the immunologic basis of these reactions.

Methods: Sera were received from 14 individuals with a history of acute systemic allergic reactions to mosquito bites, defined as the presence of one or more of the following: urticaria, angioedema, wheezing, dyspnea, hypotension, and decrease or loss of consciousness. Ten individuals were from the United States and one each was from Canada, Germany, Japan, and Switzerland. An indirect ELISA was developed to measure specific IgE and IgG antibodies to saliva from 5 common mosquito species with different geographic distributions: *Aedes aegypti*, *Aedes vexans*, *Aedes albopictus*, *Anopheles sinensis*, and *Culex quinquefasciatus*. Twenty-nine individuals with negative bite test results from laboratory-reared mosquitoes served as control subjects.

Results: Mosquito saliva-specific IgE levels to all 5 species were significantly increased in the individuals with systemic allergic reactions compared with the control subjects ($P < .061$ for *Aedes vexans* and $P < .008$ for the remaining 4 species). By using the mean of the control subjects plus 1 SD as a cut-off level, 11 individuals had positive results to *Aedes albopictus* and up to 4 additional species; 3 individuals had positive results to only one species. Saliva-specific IgG levels were not significantly increased in the individuals with systemic allergic reactions compared with levels seen in the control subjects ($P > .05$).

Conclusion: Acute systemic allergic reactions to mosquito bites involve mosquito saliva-specific IgE and can be characterized immunologically. *Aedes albopictus* is the most common species associated with systemic allergic reactions to mosquito bites. (J Allergy Clin Immunol 2004;114:1189-94.)

Key words: Acute systemic allergic reaction, *Aedes aegypti*, *Aedes albopictus*, *Aedes vexans*, *Anopheles sinensis*, anaphylaxis, angio-

edema, *Culex quinquefasciatus*, mosquito allergy, mosquito saliva-specific IgE

For most people, mosquito bites are an itchy nuisance. The small local reactions, such as wheals, flares, and delayed pruritic papules, which occur commonly during natural sensitization and desensitization to mosquito saliva, should not be referred to as mosquito allergy.¹ In some individuals, however, mosquito bites cause severe large or atypical vesicular, necrotic, or ecchymotic local allergic reactions at the bite sites²⁻⁴ or, less commonly, acute systemic allergic reactions, including urticaria, angioedema of the skin or mucous membranes (including the larynx), wheezing, dyspnea, hypotension, or decrease or loss of consciousness.⁵⁻¹⁰

There are more than 3000 mosquito species worldwide. *Aedes aegypti* saliva contains at least 31 proteins, the cDNA sequences of which have been deposited into the Genbank.¹¹ However, protein visualization techniques with gel electrophoresis and silver staining have revealed only about 20 peptides in the saliva of adult *Aedes aegypti*.¹² By using immunoblots with sera from individuals with mosquito allergy, at least 8 allergens have been found in *Aedes aegypti* saliva, and more than 16 allergens have been found in *Aedes albopictus* saliva.^{13,14} Individuals with no previous exposure to a particular mosquito species are not sensitized to the salivary antigens of the mosquito species and do not have increased mosquito saliva-specific IgE or IgG levels or skin reactivity to bites from mosquitoes of this species.^{15,16} In contrast, in response to exposure to mosquitoes and bites from mosquitoes, individuals with mosquito allergy have serum mosquito saliva-specific IgE, which can be identified with passive cutaneous anaphylaxis transfer tests (Prausnitz-Kustner tests)¹⁷; immunoassays, including ELISA^{18,19} and RAST²⁰; histamine release from basophils^{14,17}; and immunoblot techniques.^{21,22} These sensitized individuals might experience local or systemic IgE-mediated allergic reactions to mosquito saliva on re-exposure to mosquitoes. Serum mosquito saliva-specific IgE levels correlate with mosquito bite-induced immediate wheals and flares.^{19,23} IgG involvement in the pathophysiology of mosquito allergy has also been reported,^{3,14,16,23} and increases in serum mosquito saliva-specific IgG levels correlate with the size of local reactions to mosquito bites and with mosquito saliva-specific IgE levels.^{19,23} T lymphocyte-mediated delayed

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TABLE I. Geographic distribution of mosquito species used in the ELISAs for measuring saliva-specific IgE and IgG levels in sera from individuals with systemic allergic reactions to mosquito bites and control subjects

Mosquito species	Geographic distribution
<i>Aedes aegypti</i>	Worldwide cosmopolitan, Middle East, Africa, and South America
<i>Aedes albopictus</i>	North America, South America, Australia, and throughout the Orient
<i>Aedes vexans</i>	North America, Eurasia, and Africa
<i>Anopheles sinensis</i>	Throughout Eastern Asia, Japan, China, Indochina, Thailand, Siberia, and India
<i>Culex quinquefasciatus</i>	Cosmopolitan: Europe, Africa, southern United States, Asia, and South America

hypersensitivity reactions to mosquito bites might also occur.^{19,23}

In the few case reports of systemic allergic reactions to mosquito bites published to date,⁵⁻¹⁰ mosquito allergy has been investigated by using skin tests or RASTs with mosquito whole-body extracts, which contain few salivary proteins and large amounts of extraneous proteins.²⁴ Using mosquito saliva and salivary gland extracts, we have developed sensitive and specific ELISAs to measure saliva-specific IgE and IgG antibodies.¹⁸ In the present study of 14 individuals with systemic allergic responses to mosquito bites, we analyzed these saliva-specific IgE and IgG responses to 5 common mosquito species with different geographic distributions.

METHODS

Serum samples

Fourteen unsolicited serum samples from individuals with systemic allergic reactions to witnessed mosquito bites and accompanying written clinical descriptions of the reactions were received from physicians in the United States, Canada, Germany, Japan, and Switzerland during a 7-year period. Systemic allergic reactions were defined as one or more of the following symptoms and signs after a mosquito bite: urticaria, angioedema, wheezing, dyspnea, hypotension, or decrease or loss of consciousness. Control sera were obtained from 29 individuals who lived in Manitoba, Canada, and who had a negative bite test result from disease-free, laboratory-reared *Aedes aegypti* mosquitoes; all these individuals had given written informed consent for participation in a previously described University of Manitoba Research Ethics Board–approved study.²³

Mosquito salivary preparations

Saliva extracts from *Aedes aegypti* and *Aedes vexans* and salivary gland extracts from *Culex quinquefasciatus* were prepared in our laboratory.¹³ Salivary gland extracts from *Aedes albopictus* and *Anopheles sinensis* were provided by Shanghai Medical University (Dr Sulan Liu in the Department of Medical Parasitology). The geographic distribution of the 5 species studied is shown in Table I.

Briefly, mosquito saliva was collected by placing female mosquitoes in water-filled test tubes, shaking gently to temporarily immobilize them, and then confining them to the wall of a plastic box by placing Vaseline on the legs and wings. Proboscises were inserted into capillary tubes filled with 20 μ L of water, and salivation

was induced by applying 0.5% malathion in acetone (vol/vol) to the thoraces. One hour later, the contents of the capillary tubes were collected, pooled, and lyophilized. The saliva was reconstituted by dissolving the lyophilized proteins in 0.02 mol/L PBS (pH 7.2) before use. Salivary gland extracts were prepared by dissecting the heads and thoraces from mosquitoes using a binocular microscope and immediately transferring them to PBS on ice, after which they underwent ultrasound for 30 seconds and were centrifuged at 8820g for 15 minutes. The supernatant was stored at -70°C . The protein concentrations of these saliva and salivary gland preparations ranged between 0.07 and 0.40 mg/mL.

ELISAs

Indirect ELISAs¹⁸ were used to determine the concentrations of mosquito saliva-specific IgE and IgG antibodies in the sera from patients and control subjects. By using a reference serum obtained by mixing sera from individuals with mosquito allergy and increased saliva-specific IgE and IgG levels, ELISA results among assays were standardized. After choosing optimal conditions by using checkerboard titration, microtiter plates were coated with mosquito salivary preparations (about 0.05 μ g per well) overnight. Nonspecific binding was blocked with PBS–Tween 20 containing 1% BSA (Sigma Aldrich Canada, Ltd, Oakville, Ontario, Canada). Coated and blocked plates were then incubated with sera from subjects with mosquito allergy (1:20 dilution for the IgE assay and 1:80 dilution for the IgG assay). After IgE or IgG binding, the plates were treated with goat anti-human IgE (1:2,000, a gift from Dr N.F. Adkinson, Johns Hopkins University) or anti-human IgG (1:5,000, Jackson ImmunoResearch Laboratories, Inc, West Grove, Pa), followed by incubation with alkaline phosphatase-conjugated rabbit anti-goat IgG (1:5,000, Jackson ImmunoResearch). Finally, the plates were incubated with the substrate, and absorbencies were read at 410 nm. All serum samples were assayed in duplicate.

Data analysis

Analysis of data was performed with SAS software (SAS Institute, Carey, NC). For group comparisons, pooled equal variance *t* tests were used, and χ^2 tests were used to compare the positive percentages between groups.

RESULTS

Clinical data

The acute systemic allergic reactions occurred in children and young adults after one (individuals 3 and 14) or more mosquito bites. The reactions had a rapid onset (minutes) and a rapid resolution (hours). Symptoms involved urticaria or angioedema alone in 3 of the individuals (individuals 2, 3, and 9) and one or more of the following additional symptoms and signs in 11 individuals: dyspnea, wheezing, nausea, vomiting, hypotension, or decrease or loss of consciousness (Table II). Some individuals (nos. 1, 4, 5, and 11) had more than 1 systemic reaction to mosquito bites.

Here are 3 examples of patients: After 2 mosquito bites, individual 12 experienced urticaria and angioedema within 15 minutes and collapsed, with a blood pressure of 80/50 mm Hg 1 hour later. Within 5 minutes after one mosquito bite on the upper lip, individual 14 had significant swelling at the bite site and on the face and quickly became very dizzy and nearly lost consciousness. Individual 2 experienced diffuse pruritus and lip swelling,

TABLE II. Demographics of 14 individuals with systemic allergic reactions to mosquito bites

Individual no.	Age (y)	Sex	Systemic symptoms-signs	Country	Visitor–new arrival
1	12	F	Urticaria, wheezing	US (MN)	No
2	7	M	Angioedema, urticaria	US (MN)	No
3	3	F	Angioedema	US (VA)	No
4	14	F	Anaphylactic reactions	US (NC)	No
5	5	M	Urticaria, angioedema, nausea, vomiting	US (NC)/Syria	Yes
6	22	M	Edema, dyspnea, loss of consciousness	US (NY)	Yes
7	23	M	Urticaria, dyspnea	US (NY)	Yes
8	>18	M	Angioedema, wheezing	US	No
9	>18	M	Angioedema, urticaria	US	No
10	>18	M	Urticaria, angioedema, respiratory or presyncopal-type symptoms	Canada (ON)	No
12	32	F	Urticaria, hypotension (blood pressure 80/50)	Germany	No
13	21	F	Angioedema, loss of consciousness	Switzerland	No
14	>18	M	Angioedema, decreased consciousness	Japan	Yes

Treatment of the acute reaction included one or more of the following: epinephrine, H₁-antihistamine (usually diphenhydramine), glucocorticoid, intravenous fluid, oxygen, and inhaled β₂-adrenergic agonist. Participants 2, 6, 7, and 13 received immunotherapy with mosquito whole-body extract after their systemic allergic reaction to mosquito bites (rush immunotherapy in participants 6, 7, and 13). Participant 5 received prophylactic cetirizine.

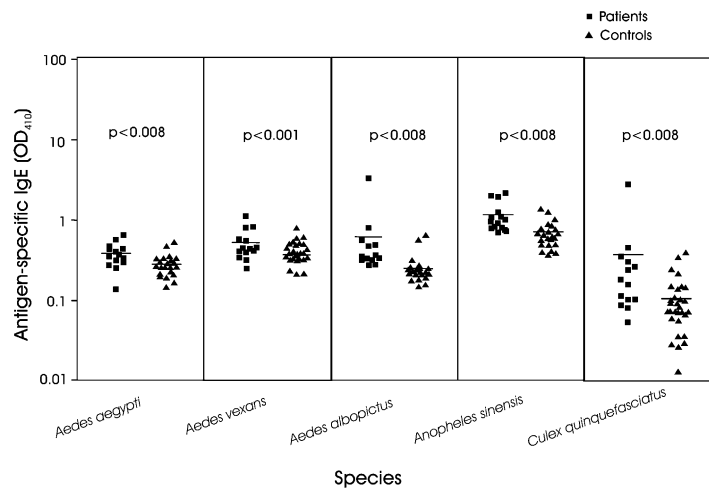


FIG 1. Mean mosquito saliva-specific IgE levels in individuals with systemic reactions to mosquito bites compared with control subjects with negative responses to laboratory-reared mosquito bites.

feeling that his throat was closing within 5 to 10 minutes after 4 to 5 mosquito bites. Individual 13 experienced local swelling, nausea, and unconsciousness within 10 to 20 minutes after mosquito bites.

Saliva-specific IgE and IgG levels to 5 mosquito species

The geometric mean levels of saliva-specific IgE to all 5 mosquito species were significantly higher in the individuals with systemic allergic reactions than in the control subjects when compared by using the Student *t* test (*P* < .061 for *Aedes vexans* and *P* < .008 for the remaining 4 species, Fig 1). The mean levels of saliva-specific IgG were also higher in the individuals with systemic allergic

reactions than in the control subjects; however, the difference was not statistically significant (*P* > .05).

In addition, IgE levels in individuals with systemic allergic reactions and control subjects were compared by using the χ² test, with the geometric mean plus 1 SD or 2 SDs of the control subjects as a positive cut-off level (Table III and Fig 2). The mean plus 1 SD of the negative control subjects was chosen because it provided the clearest differentiation between the 2 groups; results were similar to those found with the *t* test. The positive percentages of IgE were significantly higher in the individuals with systemic allergic reactions than in the control subjects (*P* < .0073, except for *Aedes vexans* [*P* = .097]; Fig 2). In contrast, the individuals with

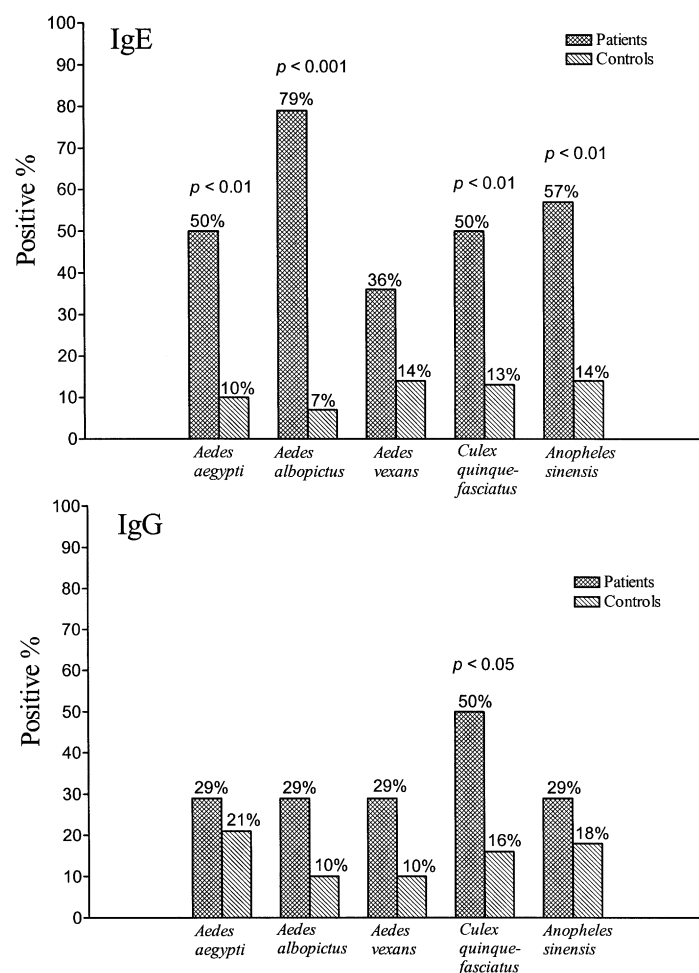


FIG 2. Percentage of positivity of serum mosquito saliva-specific IgE and IgG in individuals with systemic allergic reactions and control subjects. Positivity was determined on the basis of the geometric mean of negative control subjects plus 1 SD.

systemic allergic reactions were not more likely to have positive IgG levels ($P > .129$), except for IgG to *Culex quinquefasciatus* ($P = .0174$).

By using the mean of the control subjects plus 1 SD as a cut-off level, 11 individuals had increased IgE levels to *Aedes albopictus* and up to 4 additional species (Table III). The remaining 3 individuals had increased IgE or IgG levels to only one species: *Culex quinquefasciatus* (individual 8 from the United States), *Aedes aegypti* (individual 9 from the United States), and *Anopheles sinensis* (individual 14 from Japan).

DISCUSSION

Acute systemic allergic reactions to mosquitoes and other biting insects appear to be uncommon, although they are probably underdiagnosed and underreported. Here we describe the immune responses to mosquito saliva in 14 individuals with a clear history of such reactions. Both the mean level and the percentage of positivity for mosquito

saliva-specific IgE were significantly higher in these individuals than in the control subjects. The mean level and the percentage of positivity for mosquito saliva-specific IgG were not significantly increased in the individuals with systemic allergic reactions. In contrast, individuals with only local reactions to mosquito bites have significant increases of both saliva-specific IgE and IgG levels; in addition, the levels correlate with each other and with skin reaction size.^{3,16,23}

Some control sera from individuals with a negative mosquito bite test result contained measurable mosquito saliva-specific IgE, perhaps attributable to the cross-reactivity of mosquito salivary allergens with allergens from other biting insects and from crustaceans.²⁵⁻²⁸ Moreover, there are rare reports of cross-reactivity with allergens in Hymenoptera venom.²⁹ In addition, because the control subjects were defined on the basis of their negative bite test results to *Aedes aegypti*, the possibility exists that they might have had positive bite test results from other mosquito species if such tests had been performed.

TABLE III. Positivity of IgE and IgG antibodies to saliva from 5 mosquito species in 14 individuals with acute systemic allergic reactions to mosquito bites

Participant no.	<i>Aedes aegypti</i> (IgE/IgG)		<i>Aedes vexans</i> (IgE/IgG)		<i>Aedes albopictus</i> (IgE/IgG)		<i>Anopheles sinensis</i> (IgE/IgG)		<i>Culex quinquefasciatus</i> (IgE/IgG)	
	Mean + 1 SD	Mean + 2 SD	Mean + 1 SD	Mean + 2 SD	Mean + 1 SD	Mean + 2 SD	Mean + 1 SD	Mean + 2 SD	Mean + 1 SD	Mean + 2 SD
1	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/-	+/+	-/+
2	-/-	-/-	+/+	+/-	+/+	+/-	-/-	-/-	-/+	-/-
3	+/-	+/-	+/+	+/-	+/+	+/+	+/+	+/-	-/-	-/-
4	-/-	-/-	-/-	-/-	+/-	-/-	+/-	-/-	+/+	-/-
5	+/+	+/-	-/-	-/-	+/-	+/-	+/-	+/-	+/+	+/+
6	+/-	-/-	+/-	-/-	+/-	-/-	+/-	-/-	-/+	-/-
7	-/-	-/-	-/-	-/-	+/+	+/-	+/+	-/-	+/-	-/-
8	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	+/-	+/-
9	+/+	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
10	+/+	-/-	-/+	-/+	+/-	-/-	-/-	-/-	-/-	-/-
11	-/-	-/-	-/-	-/-	+/-	-/-	+/-	-/-	-/-	-/-
12	+/-	-/-	+/-	-/-	+/+	-/+	-/+	-/-	+/+	-/-
13	-/-	-/-	-/-	-/-	+/-	-/-	-/-	-/-	+/+	-/-
14	-/-	-/-	-/-	-/-	-/-	-/-	+/-	-/-	-/-	-/-

Positive cut-off level was determined on the basis of geometric mean of negative control subjects plus 1 or 2 SD.

On the other hand, some allergic individuals did not have an increased IgE titer to some of the mosquito species studied. This might be due to the time elapsed between the mosquito bite-induced systemic reactions and the collection of blood samples because saliva-specific IgE and IgG levels decrease over time. In one of our previous studies, the levels of mosquito saliva-specific IgE and IgG1 and IgG4 subclasses decreased significantly after the summer season.³ For example, in individual 14, who had a positive IgE titer to only one mosquito species at the cut-off level of mean plus 1 SD, the blood sample was collected 3 months after the onset of the systemic reaction.

This study provides confirmation of the existence of species-specific and species-shared allergens in mosquito saliva, as previously reported.^{13,14,30,31} The existence of species-specific allergens was indirectly confirmed in 3 individuals whose sera only reacted to mosquito saliva from one species. The existence of shared allergens in mosquito saliva was confirmed in the 8 individuals who had positive results to *Anopheles sinensis*, only 1 of whom had lived in the Orient, because *Anopheles sinensis* is found only in the Orient. *Aedes albopictus* was shown to have the most common species-shared allergens because 11 of the 14 individuals with systemic allergic reactions to mosquito bites had a positive IgE level to saliva from *Aedes albopictus*, which contains at least 16 allergens, the highest number in any of the 10 mosquito species studied to date.²¹

Mosquito salivary glands and mosquito saliva are technically difficult and time consuming to obtain. Commercially available mosquito whole-body extracts contain many extraneous proteins that are not present in mosquito saliva and are not useful in diagnostic skin tests or *in vitro* tests in individuals with a history of allergic reactions to mosquito bites.^{24,32} One example of this is the

UniCAP test, which uses mosquito whole-body extract as the capture antigen and is not as sensitive and specific (more likely to result in false-negative and false-positive results) than the ELISAs used in the present study, in which mosquito salivary extracts were used.³² In a recent study positivity was 44% for the ELISA and 25% for the UniCAP in the individuals with mosquito allergy, and 0% for the ELISA and 3% for the UniCAP in the negative control subjects.³² It is noteworthy that individuals 7 and 12 in the present study, who had negative skin test results with a mosquito whole-body extract, had positive mosquito saliva-specific IgE antibodies when measured with our ELISAs. Lack of a widely available, sensitive, specific, and safe test for determination of sensitization to mosquito saliva remains a major obstacle to accurate diagnosis of mosquito allergy and to investigation of the underlying immunologic mechanisms. Molecular cloning of mosquito saliva proteins has provided a powerful research tool to produce pure mosquito salivary allergens on a large scale for the diagnosis and treatment of mosquito allergy with standardized, effective, and safe allergens.¹⁴ Currently, 4 recombinant *Aedes aegypti* salivary allergens (rAed a 1, rAed a 2, rAed a 3, and rAed a 4) have been expressed and identified.³³⁻³⁷ Immunoassays with these recombinant allergens are being developed.³⁸

In summary, we describe the humoral immune response to mosquito saliva allergens in individuals with acute systemic allergic reactions studied by using sensitive and specific immunoassays. Mosquito saliva-specific IgE, but not IgG, was found to be important in systemic allergic reactions to mosquito bites, and both species-specific and species-shared allergens were identified in individuals with systemic allergic reactions. In the future, recombinant mosquito salivary allergens will be useful for

confirming the diagnosis of mosquito allergy and for specific immunotherapy in persons with severe systemic allergic reactions to mosquito bites.

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Correction

With regard to the November 2004 article entitled "Immune responses to mosquito saliva in 14 individuals with acute systemic allergic reactions to mosquito bites" (2004;114:1189-94): In Table II (page 1191), the data pertaining to individual no. 10 were presented incorrectly and no. 11 was missing. A correct version of the Table is presented here:

TABLE II. Demographics of 14 individuals with systemic allergic reactions to mosquito bites

Individual no.	Age (y)	Sex	Systemic symptoms-signs	Country	New arrival
1	12	F	Urticaria, wheezing	US (MN)	No
2	7	M	Angioedema, urticaria	US (MN)	No
3	3	F	Angioedema of face	US (VA)	No
4	14	F	Anaphylactic reactions	US (NC)	No
5	5	M	Urticaria, angioedema, nausea, vomiting	US (NC)/Syria	Yes
6	22	M	Angioedema, dyspnea, loss of consciousness	US (NY)	Yes
7	23	M	Urticaria, dyspnea	US (NY)	Yes
8	>18	M	Angioedema, wheezing	US	No
9	>18	M	Angioedema, urticaria	US	No
10	>18	M	Reported as anaphylactic reaction	US	No
11	29	M	Urticaria, angioedema, respiratory and presyncopal-type symptoms	Canada (ON)	No
12	32	F	Urticaria, hypotension (blood pressure 80/50)	Germany	No
13	21	F	Angioedema, loss of consciousness	Switzerland	No
14	>18	M	Angioedema, decreased consciousness	Japan	Yes

Treatment of the acute reaction included one or more of the following: epinephrine, H₁-antihistamine (usually diphenhydramine), glucocorticoid, intravenous fluids, oxygen, and inhaled β_2 -adrenergic agonist. Participants 2, 6, 7, and 13 received immunotherapy with mosquito whole-body extract after their systemic allergic reaction to mosquito bites (rush immunotherapy in participants 6, 7, and 13). Participant 5 received prophylactic cetirizine.