

Passive transfer of cutaneous mosquito-bite hypersensitivity by IgE anti-saliva antibodies

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Abstract

TOP

Background: Mosquito bites frequently cause cutaneous wheal and flare reactions, and recent immunoblotting studies have shown specific anti-saliva IgE antibodies in many persons who have such reactions.

Objective: The study was designed to show that human serum containing mosquito saliva-specific IgE antibodies can produce histamine release in vitro and whealing in vivo.

Methods: Two mosquito bite-tolerant subjects had bite challenges and Prausnitz-Küstner tests with heated and unheated serum from one patient with *Aedes* mosquito allergy. Immunoblotting and basophil histamine release tests were performed with the patient's and subjects' sera.

Results: Both mosquito bite-tolerant subjects had positive Prausnitz-Küstner reactions, which indicated a successful transfer of cutaneous mosquito hypersensitivity. The ordinary and passive basophil histamine release tests also produced positive results with *Aedes communis* antigens.

Conclusion: The results of the Prausnitz-Küstner test, immunoblotting, and basophil histamine release tests are consistent with the hypothesis that mosquito bite whealing is mediated by specific anti-saliva IgE antibodies. (J ALLERGY CLIN IMMUNOL 1994;94:902-6.)

BHRT Basophil histamine release test

P-K Prausnitz-Küstner

WBE Whole-body extract

Keywords: [Mosquito allergy](#), [IgE and IgG₄ antibodies](#), [Prausnitz-Küstner test](#); [basophil histamine release test](#)

Mosquito bites cause cutaneous wheal and flare reactions and delayed bite papules in most persons, but in contrast to bee and wasp stings, anaphylactic reactions are extremely rare.^{1,2} Evidence that whealing and the rare immediate systemic symptoms caused by mosquito bites are mediated by IgE antibodies, as they are in Hymenoptera and other insect allergies, is increasing. Studies in animals and human beings have demonstrated successful passive transfer of immediate mosquito hypersensitivity.³⁻⁵ A few studies have detected IgE antibodies to mosquitoes in human sera by using whole body extract (WBE) or salivary gland extracts as antigens.^{6,7} We have developed a sensitive immunoblotting assay in which mosquito saliva is used as antigen, and we have shown that most subjects with immediate hypersensitivity to *Aedes communis* bites have IgE antibodies against the 36 kd and 22 kd saliva proteins.⁸

In this study we performed passive transfer experiments with basophil histamine release and Prausnitz-Küstner (P-K) tests to show that human serum containing mosquito saliva-specific IgE antibodies can produce histamine release in vitro and whealing in vivo.

Methods

Subjects

The patient with mosquito allergy was a 12-year-old boy who had experienced severe mosquito bite reactions since the age of 2 years. At this age, mosquito bites caused large wheals accompanied several times by systemic symptoms such as generalized urticaria and mild wheezing. These symptoms responded well to administration of epinephrine and intramuscular hydrocortisone. >From the age of 5 years, whealing was somewhat milder, but the delayed bite reactions were more severe. Large erythematous swellings, often accompanied by malaise and mild fever, developed 2 to 12 hours after the bites and persisted for several days.

When the patient was 10 years old, bite challenges with *A. communis* mosquito, a species that is most prevalent in the forests of southern Finland, and with *A. aegypti* mosquito, a related tropical and subtropical species, confirmed his mosquito allergy. Challenges performed with cetirizine (0.2 mg/kg) showed 7 to 8 mm diameter bite lesions at 15 minutes, 13 to 16 mm lesions at 2 hours, and 75 to 90 mm lesions at 24 hours. An in-house and a commercial (Pharmacia, Uppsala, Sweden) RAST with WBE of *A. communis*, however, produced negative or borderline (0.35 kU/L) results.

The two mosquito bite-tolerant subjects were a 49-year-old physician (recipient 1) and a 52-year-old nurse (recipient 2). Both had been exposed to local mosquitoes for many years and had also visited countries where *A. aegypti* mosquitoes are prevalent. Both were nonreactive (no immediate or delayed bite reactions) in the field, and they showed negative responses to *A. communis* and minute whealing in response to *A. aegypti* bite challenges. The result of a BHRT with *A. communis* WBE antigen was also negative (histamine release < 10%) in both recipients.

Mosquitoes and P-K test

Adult *A. communis* mosquitoes were raised from larvae collected in southern Finland. The *A. aegypti* mosquitoes were derived from a strain kept for many years in the insectary of the Laboratory of Protozoology, Institute of Tropical Medicine in Antwerpen, Belgium. Three mosquitoes were put in a small cage, which was placed on the volar side of each recipient's forearm. After the bites, the size of wheal and delayed bite reactions were measured in millimeters at 15 minutes and at 1, 2, 6, 12, and 24 hours.

The P-K test was performed with unheated and heated (4 hours at 56° C) patient serum administered to both recipients. Serum (0.05 ml; diluted 1:2, 1:5, 1:10, 1:100 in phosphate-buffered saline) was injected intradermally in the forearms. Controls included unheated recipient serum (negative for human

immunodeficiency virus and hepatitis antibodies) and phosphate-buffered saline. A mosquito bite challenge was performed exactly at the injection sites after 24 hours, and at the same time a histamine control (10 mg/ml; ALK A/S, Horsholm, Denmark) was performed with a prick test lancet. Because the bigger *A. communis* mosquitoes did not bite when in the cage, the P-K test had to be performed only with *A. aegypti* mosquitoes.

Demonstration of IgE and IgG₄ anti-saliva antibodies by immunoblotting

WBE of *A. communis* mosquitoes was prepared, and the saliva was collected as previously described.⁸ The protein concentrations measured by the Bradford method were 14 mg/ml for the WBE and 0.08 mg/ml for the saliva antigen in the stock solutions.

An immunoblotting method was used to study IgE and IgG₄ anti-saliva antibodies in the patient and recipient sera as previously described.⁸ In brief, the saliva samples were electrophoresed in sodium dodecylsulfate–polyacrylamide gels and electroblotted onto polyvinylidene difluoride membranes. The serum samples (diluted 1:4 for IgE and 1:10 for IgG₄) were incubated with biotinylated goat anti-human IgE (1:250; Vector, Burlington, Calif.), with biotinylated mouse monoclonal anti-human IgG₄ (1:250; Zymed, San Francisco, Calif.), or with mouse monoclonal anti-human IgE (1:250; Zymed) followed by biotinylated rat monoclonal anti-mouse IgM (1:250; Zymed). The strips were then stained with alkaline phosphatase–conjugated streptavidin (1:1000, Bio-Rad, Richmond, Calif.) and its substrate. Rabbit serum immunized with *A. communis* bites was used as control.

BHRTs

The BHRT was performed with *A. communis* WBE and saliva antigens. Washed whole blood cell suspensions were incubated with serial dilutions of antigens or with medium only at 37° C for 30 minutes. The histamine contents of the supernatants were measured by radioenzymatic assay with histamine-N-methyl-transferase.⁹ The detection limit was 0.1 ng/ml, and the results were expressed as the percentage of released histamine of total blood histamine.

A passive BHRT was performed with the cells of recipient 1. Heparinized blood was drawn immediately before P-K testing. The cells washed with piperazine-N,N'-bis[2-ethanesulfonic acid] calcium buffer, pH 7.4, were incubated at 37° C for 2 hours with unheated or heated patient serum (diluted 1:5) and with the control serum from recipient 2. After incubation, the cells were washed with piperazine-N,N'-bis[ethanesulfonic acid] calcium buffer, and the BHRT was performed as described above with serial dilutions (1:600, 1:6000, 1:60,000) of *A. communis* WBE and saliva.

Results

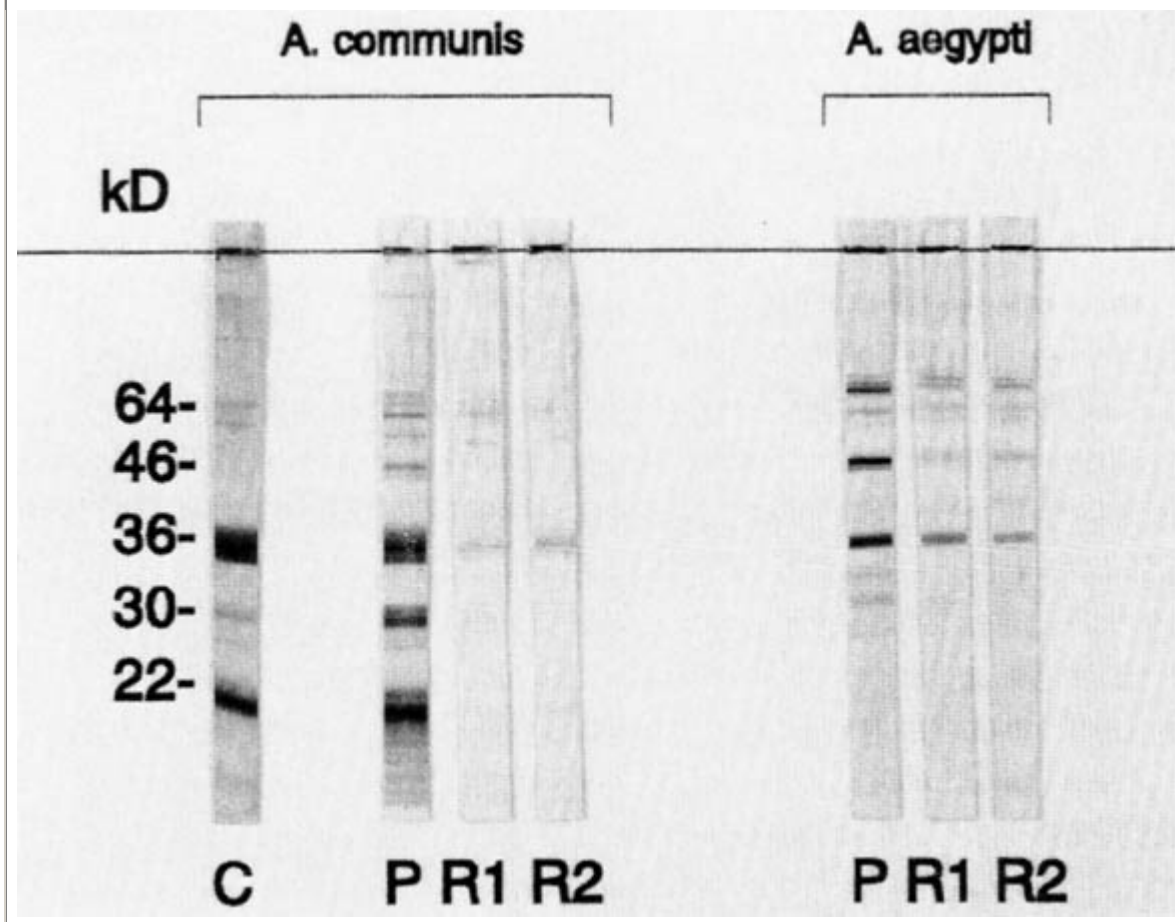
P-K test

Results of the P-K test were positive in both recipients. In recipient 1 mosquito bites caused 5 mm diameter wheals at transferred serum sites (dilutions of 1:2 and 1:10), and in recipient 2 the diameters were 14 mm (dilution of 1:5) and 4 mm (dilution of 1:100). With heated patient serum, the 15-minute reaction was negative (2 mm) in recipient 1 and clearly diminished (diameter of 4 mm, dilution of 1:5) in recipient 2. The saline and control serum sites also showed minute wheals (diameter of 2 mm), which were clearly smaller than those caused by histamine (diameters of 5 and 6 mm). In addition to immediate reactions, the recipients showed delayed bite lesions (diameters from 2 to 12 mm), which persisted for up to 6 hours, but at 12 hours the challenge sites were negative.

Immunoblotting

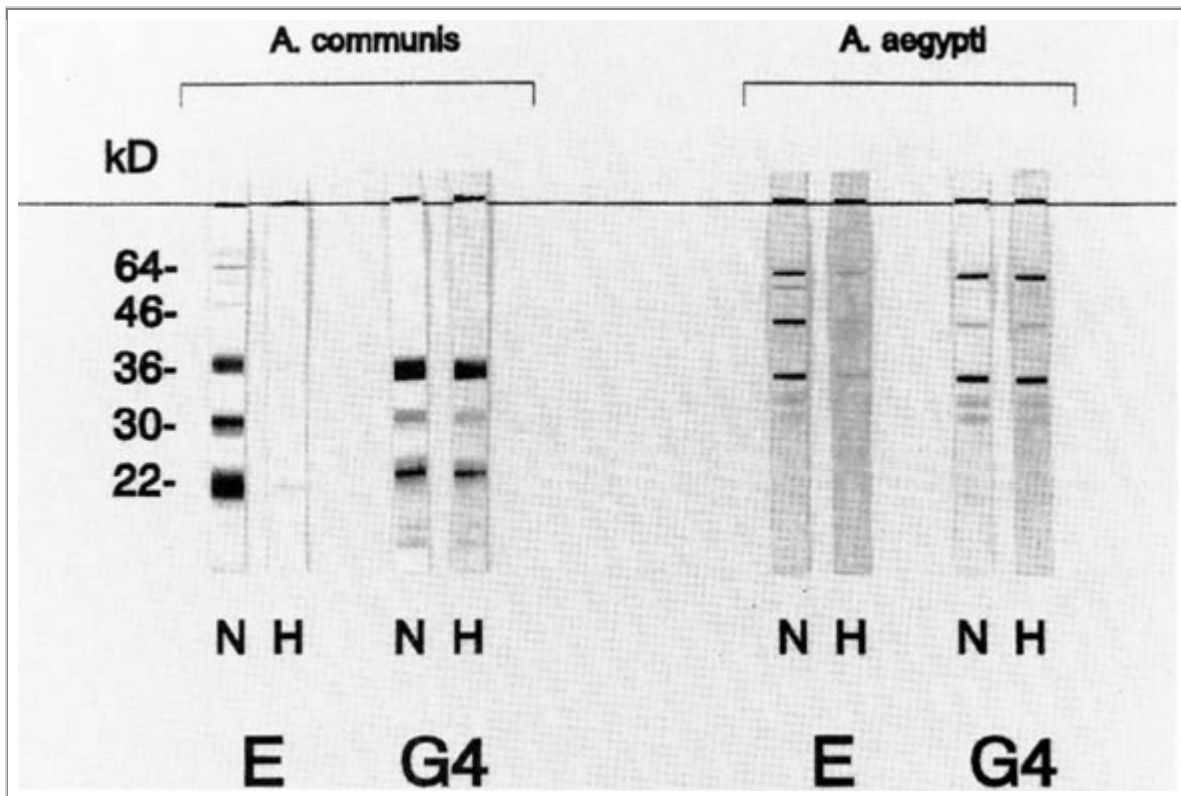
The patient serum showed clear IgE bands in response to 36, 30, and 22 kd *A. communis* and to 46 and 36 kd *A. aegypti* saliva antigens (■Fig. 1). The two recipient sera demonstrated only very faint IgE bands in response to 36 kd *A. communis* and more intense bands in response to 36 kd *A. aegypti* antigens (■Fig. 1).

FIG. 1. The serum from the patient with mosquito allergy (P) shows intense IgE antibody bands in response to the major *A. communis* and *A. aegypti* saliva antigens in immunoblotting. The two recipient sera (R1 and R2) shows very faint IgE bands in response to *A. communis* and more intense bands in response to *A. aegypti* saliva antigens. A control serum (C) from a rabbit immunized with 60 *A. communis* bites shows an IgG antibody response against the major 22, 30, and 36 kd saliva antigens.



Heat treatment of the patient serum caused almost total disappearance of the three major IgE bands that responded to *A. communis* and *A. aegypti* saliva antigens (■Fig. 2). In contrast, heat treatment did not reveal any major changes in the intensity of IgG₄ bands (■Fig. 2).

FIG. 2. Effect of heat treatment on the anti-saliva antibodies in the serum from the patient with mosquito allergy. IgE antibodies, but not IgG₄ antibodies, to *A. communis* and *A. aegypti* saliva antigens disappear almost completely from the heated serum (H) compared with unheated serum (N).



BHRTs

In ordinary BHRTs with patient cells all three dilutions of *A. communis* WBE induced a positive histamine release (87% with 1:600 dilution, 86% with 1:6000 dilution, and 58% with 1:60,000 dilution). When saliva was used as antigen, the histamine releases were 82%, 39%, and 15%, respectively.

Passive BHRT showed histamine release from the recipient cells when they had been treated with the patient serum and challenged with WBE of *A. communis*. The 1:600 dilution caused 78% histamine release, and the 1:6000 dilution caused 25% histamine release. The result with 1:60,000 dilution of WBE was negative, as were the results with saliva. The results of controls with heated patient and unheated recipient 2 sera were also negative.

Discussion

In this study the P-K test showed a successful transfer of cutaneous mosquito hypersensitivity with the serum from an allergic patient to two nonreactive recipients. The challenges were performed as naturally as possible (i.e., with *A. aegypti* mosquito bites). Heated patient serum did not transfer whealing, and in agreement with this, immunoblotting confirmed that the saliva-specific IgE antibodies were heat-labile. Moreover, the passive BHRT performed with basophils from a nonreactive subject and serum from the patient with mosquito allergy confirmed the successful passive transfer in vitro.

A few points in these experiments and their results require comment. In the P-K test the challenges had to be performed with *A. aegypti* mosquitoes, although the patient had not been exposed to this species in the field. On the other hand, in passive BHRT we could use only *A. communis* antigens because sufficient saliva was not available from *A. aegypti*. However, the fact that the patient reacted to *A. aegypti* bites, had IgE antibodies reacting both against *A. aegypti* and *A. communis* saliva antigens, and showed a positive BHRT response to *A. communis* saliva strongly suggests that the anti-*Aedes* mosquito antibodies are cross-reactive.

A previous study with a P-K test and *A. aegypti* bite challenges demonstrated successful passive transfer of immediate response in one of the 10 recipients.⁵ The P-K test results in this study, although performed with

the serum of only one patient with mosquito allergy, also strongly suggest that the frequently occurring anti-saliva IgE antibodies [8](#), [10](#) are important in the pathogenesis of immediate cutaneous hypersensitivity to mosquitoes. In support of this, the present immunoblotting experiments showed heat-labile saliva-specific IgE antibodies in the transferred patient serum. In the P-K test we also found positive reactions at 1 and 6 hours. Because the 15-minute reaction was caused in all likelihood by specific IgE antibodies, these delayed reactions could well be cutaneous late-phase reactions.

In this study both the patient and recipient sera showed IgG₄ type antibodies to mosquito saliva antigens. The IgG₄ antibodies were directed mainly against the same saliva proteins as the IgE antibodies. As expected, the IgG₄ antibodies were heat-resistant and obviously were not involved in the transfer of immediate hypersensitivity in the P-K test. The absence of IgE but presence of IgG₄ anti-saliva antibodies in the sera of the two recipients exposed for a long time to mosquitoes and now tolerant to *A. communis* bites suggests that the IgG₄ anti-saliva antibodies could be markers of natural desensitization to the bites.

In conclusion, these P-K test, immunoblotting, and BHRT results are consistent with the hypothesis that mosquito bite whealing is mediated by specific anti-saliva IgE antibodies.

We thank S. Mäkinen-Kiljunen, MSc, for performing the RAST studies and Mrs. M. Karsten for the excellent secretarial work. We also thank Mrs. A. Correwyn and Mr. G. Timperman for rearing *A. aegypti*.

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- Received for publication Oct. 21, 1993.
- Revised May 9, 1994. .
- Accepted for publication May 19, 1994.
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- 0091-6749/94 \$3.00 + 0 **1/1/57698**

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Journal of Allergy and Clinical Immunology, The
August 1996 • Volume 98 • Number 2

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