

Histamine and leukotriene C₄ release in cutaneous mosquito-bite reactions

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Background: Mosquito bites are known to sensitize persons, and the most common cutaneous reactions are immediate wheals and delayed bite papules. Anti-saliva IgE and IgG₄ antibodies are common in mosquito-sensitive subjects, but mediator release in bite reactions is not known.

Methods: We used the microdialysis technique to measure in vivo histamine and leukotriene C₄ release after bite challenges in six mosquito-sensitive subjects. One individual who was not sensitive to bites volunteered as a control subject.

Results: Three of the six mosquito-sensitive subjects had large wheals and showed clearly increased histamine concentrations 30 to 45 minutes after the bites. The histamine levels declined to baseline value within 2 hours; thereafter, one subject showed a second increase in histamine concentration. Four of the six mosquito-sensitive subjects showed increased leukotriene C₄ concentrations, and this mediator seemed to be released somewhat later than histamine.

Conclusions: The increased histamine and leukotriene C₄ release observed in this study suggests that both mediators are involved in the early allergic response caused by mosquito bites. (*J Allergy Clin Immunol* 1996;98:408-11.)

Key words: *Aedes aegypti* mosquito, wheals, bite papules, mediator release

Immediate wheals and delayed bite papules are the most common cutaneous reactions to mosquito bites.¹ When feeding, mosquitoes inject saliva into the skin, and the salivary proteins can sensitize persons. Specific IgE and IgG₄ antibodies are frequently found by immunoblotting in the sera of children and adults who show wheals caused by mosquito bites.² After wheals have formed, many mosquito-sensitive individuals have delayed bite papules, which appear after 4 to 6 hours and may persist for several days.¹

Several studies suggest that histamine is an

Abbreviation used
LTC₄: Leukotriene C₄

important mediator in immediate mosquito-bite reactions. The lung tissue cells from passively sensitized guinea pigs release histamine when challenged with mosquito extract.³ Cetirizine, an H₁-blocking antihistamine, decreases mosquito-bite wheal formation and pruritus significantly.⁴ In this study we used a novel microdialysis technique⁵ and showed that histamine and leukotriene C₄ (LTC₄) are released in mosquito-bite reactions on human skin.

METHODS

Subjects

Six adult subjects sensitive to mosquito bites were studied (Table I). All subjects showed 15-minute whealing in mosquito-bite challenge. One individual who was not sensitive to mosquito bites (no immediate or delayed reactions) volunteered as a control subject. Three atopic subjects (1 man and 2 women, aged 42 to 46 years) with positive skin prick test and RAST (Pharmacia, Uppsala,

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TABLE I. Size of bite reaction and total histamine and LTC₄ release within 2 hours in six mosquito-sensitive subjects and one control subject

Subject No.	Sex	Age (yr)	Size of histamine prick (mm ²)	Size of bite reaction (mm ²)			Histamine release (pmol)		LTC ₄ release (pg)	
				15 min	60 min	120 min	60 min	120 min	60 min	120 min
1	M	36	24	20	28	13	3.0	5.9	37.3	43.3
2	F	19	ND	19	39	3	3.2	7.3	59.0	131.0
3	F	35	28	118	177	0	12.8	21.6	134.6	178.4
4	F	30	20	177	177	104	5.7	7.2	13.0	77.0
5	F	30	28	42	94	24	14.6	19.6	19.4	44.3
6	F	59	38	12	1	0	0.4	2.6	23.6	48.2
7	M	50*	20	0	0	0	0.4	0.7	10.7	19.5

ND, Not done.

*Nonreactive control subject.

Sweden) (16.25, 5.28, and 1.58 kU/L) responses to cow allergen were used as positive control subjects for histamine release. The study was approved by the Ethics Committee of the Hospital for Skin and Allergic Disease of Helsinki University.

Microdialysis equipment

The equipment included a pump (CMA/100), a probe (CMA/20), and a fraction collector (CMA/140) (CMA Microdialysis, Stockholm, Sweden). The shaft length of the probe was 25 mm, the membrane length was 10 mm, and the membrane diameter was 0.5 mm. The semipermeable membrane allows passive diffusion of small-molecular-size substances up to 20,000 d from the extracellular space into the perfusing isotonic saline solution (Ringersteril; Medipolar, Oulu, Finland). The concentration of histamine in the microdialysis perfusate versus the concentration of histamine outside the dialysis membrane is called the relative recovery. This was 35% when 20 nmol/L and 200 nmol/L histamine standards dissolved in saline solution were used.

Microdialysis and mosquito-bite challenge

Two microdialysis probes were inserted into the forearm at least 10 cm apart. Because implantation trauma can increase temporarily histamine levels,⁵ the mosquito-bite challenges were started 1 hour after the insertion of the probes. Six *Aedes aegypti* mosquitoes (a laboratory strain obtained from the Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium) were placed in a small cage and allowed to feed. The bite distance was always less than 5 mm from the probes. A control bite and histamine (10 mg/ml) prick test were performed on the contralateral forearm. The diameter of the control bite reaction was measured at 15 and 30 minutes and at 1, 2, 6, and 24 hours.⁴ In the three atopic control subjects, the skin was pricked four times, approximately 3 mm from the probe, with a lancet through a drop of cow dander extract (10 mg/ml).

A perfusion rate of 3.3 µl/min was used in the microdialysis. Samples for histamine were collected at

15-minute intervals (49.5 µl) and for LTC₄ at 30-minute intervals (99.0 µl) for up to 2 hours. In three subjects histamine collection and in two subjects LTC₄ collection was prolonged up to 4 hours. In atopic control subjects, histamine samples were collected for up to 1 hour. The samples were stored for less than 4 weeks at -20°C before analysis.

Analysis of histamine and LTC₄

Histamine was analyzed in duplicate by using a radioenzyme assay with a detection limit of 0.5 to 1.0 nmol/L.⁶ LTC₄ was analyzed by using a commercial radioimmunoassay kit (Leukotriene C4 specific [³H] assay systems; Amersham, U.K.), which required a 90 µl sample and had a detection limit of 2 pg. The radioactivity was measured by using an LKB 1215 Rackbeta liquid scintillation counter (Wallac Oy, Turku, Finland).

RESULTS

Histamine release

The mean baseline histamine concentration before mosquito-bite challenge in the six mosquito-sensitive subjects was 6.1 ± 4.2 nmol/L (mean ± SD). After the mosquito bites, the first 15-minute fraction did not show any increase in the histamine concentration (5.25 ± 4.8 nmol/L), but thereafter, a clear rise was found in the 15-to-30-minute and 30-to-45-minute fractions (33.6 ± 36.6 nmol/L and 58.7 ± 55.7 nmol/L, respectively). A slow decline was observed in the subsequent 15-minute fractions for up to 2 hours (35.9 ± 33.5 nmol/L, 26.1 ± 21.5 nmol/L, 22.8 ± 13.1 nmol/L, 20.0 ± 12.0 nmol/L, and 14.2 ± 9.0 nmol/L, respectively).

Three of six mosquito-sensitive subjects showed markedly increased histamine concentrations (maximum, 136 nmol/L), whereas the remaining three subjects showed slightly but clearly elevated levels during the 2-hour sampling period (Fig. 1). After 2 hours, one of the three subjects again

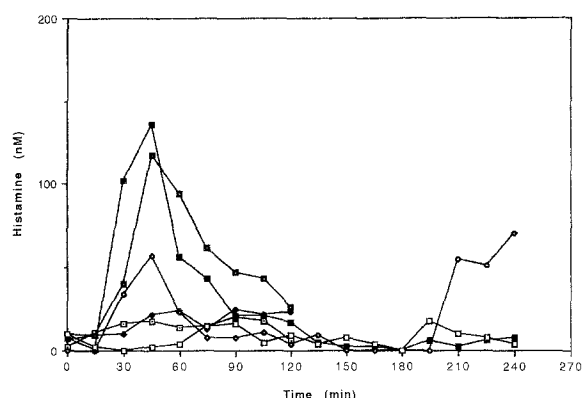


FIG. 1. Individual histamine release (in nanomoles per liter [nM]) after mosquito-bite challenge in six mosquito-sensitive subjects. In three subjects, samples were collected for up to 4 hours, and one subject showed a second increase in histamine concentration.

showed a marked increase (maximum, 70.4 nmol/L), and another subject showed a slight increase (maximum, 17.8 nmol/L) as shown in Fig. 1. In the control subject not sensitive to mosquito bites, the baseline histamine concentration was 6.9 nmol/L. After the mosquito-bite challenge, all subsequent 15-minute samples showed low histamine concentrations (from 0 to 3.6 nmol/L).

In the three atopic control subjects, the mean baseline histamine concentration before prick testing with cow allergen was 6.7 ± 4.2 nmol/L. Thereafter, the concentrations in the subsequent 15-minute fractions were 15.7 ± 6.0 nmol/L, 32.7 ± 2.5 nmol/L, 13.3 ± 3.2 nmol/L, and 4.2 ± 4.0 nmol/L, respectively.

LTC₄ release

In the six mosquito-sensitive subjects, the mean baseline LTC₄ concentration was 277.3 ± 310.7 pg/ml. After the mosquito bites, the mean LTC₄ concentration in the first 0-to-30-minute fraction was 408.24 ± 346.9 pg/ml, and in the subsequent 30-minute fractions it was 653.2 ± 1155.4 , 239.5 ± 179.9 , and 632.7 ± 548.6 pg/ml, respectively.

Three of the six subjects showed the highest LTC₄ concentrations in the 90-to-120-minute samples (1422, 1067, and 778 pg/ml, respectively). In one of the two subjects, who was followed up for 4 hours, the highest LTC₄ concentrations were found in the 150-to-180-minute (1822 pg/ml) and in the 180-to-210-minute samples (1400 pg/ml). The mosquito-bite-nonreactive control subject had low LTC₄ levels in all five samples (101, 100, 116, 88, and 90 pg/ml, respectively).

Comparison of total histamine and LTC₄ release

Table I shows the total amount of histamine and LTC₄ in collected fractions 1 and 2 hours after mosquito-bite challenge in six mosquito-sensitive subjects and one control subject. The sizes of the bite reactions at 15, 60, and 120 minutes are also given. The total amount of histamine released within 2 hours varied from 2.6 to 21.6 pmol, and the total amount of LTC₄ varied from 43.3 to 178.4 pg. The three subjects showing the largest 15-minute wheals also had the greatest histamine release within 1 hour.

DISCUSSION

In this study we used the microdialysis method to monitor histamine and LTC₄ release in mosquito-bite reactions. The microdialysis apparatus allows accurate kinetic measurements of low-molecular-weight mediators in the extracellular fluid of the skin; and, for example, histamine release can be measured after allergen or substance P challenge.^{5,7} In this study we examined mosquito-bite-sensitive subjects and could, for the first time, obtain direct evidence that histamine is released in significant quantities after the bites. The three subjects who exhibited the highest release curves also showed the largest wheal reactions. The highest histamine concentrations were found in the 30-to-45-minute fractions, and normal levels were recovered within 120 minutes. This rather slow and long-lasting histamine release after the mosquito bites was somewhat different from that observed after prick testing with cow allergen. In the latter experiment, the increase and decline of histamine were more rapid, and normal levels were reached within 60 minutes. In contrast to the allergic subjects, the control subject, who was not sensitive to the bites, demonstrated no histamine release caused by mosquito bites. In agreement with this, microdialysis measurements did not show any spontaneous histamine release in the eight control subjects studied previously without any allergen challenge by Andersson et al.⁵

All six mosquito-sensitive subjects showed 15-minute whealing, and therefore the observed early histamine release could well be due to the mast cell activation by saliva-specific IgE antibodies.^{8,9} After wheals had formed, four of the six subjects showed delayed bite reactions for up to 6 hours, and three of them, for up to 24 hours. Skin biopsy specimens from mosquito bites have shown that eosinophils and neutrophils are found in the dermis already at 2 to 6 hours.¹⁰ Because basophils

usually comigrate with the eosinophils to the site of an allergic reaction, the second histamine peak observed after 2 hours in the mosquito-sensitive subjects could be due to histamine released from the basophils. The late histamine release could also be explained by a new activation of recovered mast cells if a sufficient amount of mosquito saliva allergen is still present in the dermis.

In addition to histamine, we also observed elevated LTC₄ concentrations in four of the six mosquito-sensitive subjects, and LTC₄ seemed to be released somewhat later than histamine. No correlation could be found between individual histamine and LTC₄ concentrations, but this may be because the number of subjects was too small for direct comparisons. Mast cell activation may be a source of LTC₄ release, but eosinophils and basophils could also be involved because they are known to secrete LTC₄.¹¹

In this study we could show with microdialysis method that histamine and LTC₄ are released in mosquito-bite reactions in human skin. The mediator release was rather slow, and both histamine and LTC₄ could be observed for up to 4 hours after the bites. At present, there is more evidence that histamine and LTC₄ are released by immunologic mechanisms than by nonimmunologic mechanisms. The mosquito-bite–nonreactive control subject in this study showed no increased histamine or LTC₄ release and had no saliva-specific IgE antibodies.⁹ We have also shown with capture ELISA that the level of saliva-specific IgE antibodies correlates with the size of the mosquito-bite wheals in young children, whereas infants do not have saliva-specific IgE antibodies and are nonreactive to the bites (H. Brummer-Korvenkontio et al. Unpublished data). Finally, our passive transfer experiments have confirmed that mosquito-bite wheals are mediated by saliva-specific IgE antibodies

and that these antibodies also cause histamine release from basophils.⁹

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