

Cutaneous reactivity to mosquito bites: effect of cetirizine and development of anti-mosquito antibodies

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Summary

Cutaneous reactivity to mosquito bites was examined in 27 adult volunteers exposed to *Aedes communis* mosquitoes. Twenty-three subjects showed a combination of immediate wealing and delayed bite-papules, two subjects each experienced only immediate or delayed cutaneous reactions and two were non-responsive to the bites. The mean size of wealing and the mean score of pruritus was similar in 19 non-atopic and in eight atopic volunteers. These results confirm that normal subjects exhibit different stages of sensitization to mosquito bites. At the onset of the mosquito season, immunoblotting showed that four of 21 subjects (19%) had IgG-class antibodies to a recently described 21.5 kD *Aedes communis* mosquito antigen. After a 10-day exposure to a mean of 47 mosquito bites, these antibodies were found in 10 subjects (48%) who exhibited both strong and weak cutaneous bite-lesions. A placebo-controlled, double-blind study with cetirizine 10 mg was performed to examine the effect of this non-sedating antihistamine on mosquito bites. The bite lesions were measured and pruritus scored at 15 min, 60 min, 12 hr, and 24 hr. Cetirizine decreased significantly immediate wealing and pruritus ($P < 0.01$), but had no effect on the delayed symptoms. This result supports the view that immediate mosquito-bite reactions are histamine-mediated.

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Introduction

Mosquitoes are very numerous in the far northern areas of North America, Europe, and Asia. Certain species are also prevalent in the salt-marshes along the Atlantic coast and Gulf of Mexico, while others infest rural and urban areas [1]. Female mosquitoes need meals of blood; when feeding, they inject saliva into the skin. In man, cutaneous responses to the bites vary from common immediate weal-and-flare reactions and delayed papules to more rare bullous eruptions and Arthus-type reactions with systemic symptoms [1-4]. Various antihistamines to treat mosquito bites have been tried, but a carefully controlled study failed to show any benefit from pyribenzamine cream applied either before or after mosquito bites [5]. A few old studies suggest that antihistamines taken orally

may relieve pruritus, but otherwise do not reduce the mosquito-bite lesion [1-3,6].

The immediate mosquito-bite lesion is generally considered to be IgE-mediated, but in contrast to bee and wasp sting allergy, there is no conclusive evidence to confirm the occurrence of IgE-class anti-mosquito antibodies (AMA) [7]. The pathogenesis of the delayed mosquito bite-papules has remained even more obscure; in addition to Arthus-type mechanisms, both late-phase type I cutaneous reactions and cell-mediated immunity may be involved [7,8]. Recently, several studies have described IgG-class AMA in man, but the role of these antibodies in mosquito-bite reactions is unknown [9-12].

The objective of the present study was to characterize normal subjects' cutaneous reactivity to a common, man-biting mosquito. In addition, we performed a double-blind, placebo-controlled trial with cetirizine to investigate the role of histamine in mosquito-bite reactions. During the study we also examined the IgG-class AMA response by immunoblotting.

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Materials and methods

Subjects and mosquito exposure

Twenty-seven healthy adults, medical students, nurses, or doctors, volunteered for the study. There were 10 men and 17 women, the mean age of whom was 31 (range 18–49) years. A detailed medical history was taken, and all subjects were prick-tested with 22 common inhalant allergens (ALK A/S, Copenhagen, Denmark) to determine their atopic constitution. Twenty-one volunteers were exposed to mosquitoes at the onset of the mosquito season in late May and June in 1989. The remaining six subjects were exposed later in the season. The exposure took place in forests in southern Finland, where most of the biting mosquitoes are the *Aedes communis* species [13,14]. The 10-day exposure period included two main exposure days when the volunteers tried to get 10 bites in the late afternoon or evening. In the other days, the volunteers were encouraged to take at least a few bites. The bites were taken on the forearm, and the mosquitoes were allowed to feed to repletion. During the 10-day trial period, the 27 volunteers received a mean of 39 bites (range 6–80 bites).

Cetirizine administration and measurement of bite-reactions

After the 5-day baseline exposure period the volunteers received either cetirizine-HCl 10 mg or placebo tablets for the next 5 days. They took the tablets in the morning, and observed any possible side-effects. On the main exposure days in the baseline and treatment period the diameter of the largest bite lesion was measured at 15 min, 60 min, 12 hr, and 24 hr. The immediate 15 min weals were scored as strong (diameter > 10 mm), weak (diameter 2–10 mm) or negative (diameter < 2 mm) and the delayed 24 hr papules as strong (diameter > 5 mm), weak (diameter 2–5 mm) or negative (diameter < 2 mm). At the same time, the pruritus was scored on a visual analogue scale ranging from 0 to 100 mm. The differences between baseline and treatment values for bite lesions and pruritus between the cetirizine-treated and placebo groups were analysed by one-way analysis of variance. Four subjects whose baseline bite lesions were negative or whose baseline pruritus score was lower than 5 mm were excluded. The final analysis was thus based on the data from 11 volunteers (5 men, 6 women) receiving cetirizine and 12 (2 men, 10 women) receiving placebo. The mean weight was 69.4 kg in the cetirizine and 63.3 kg in the placebo group ($P=0.18$; student's *t*-test). The mean number of mosquito-bites in the cetirizine-treated group was 48 (range 14–80) and in the placebo group 34 (range 8–63), a non-significant difference ($P=0.11$; student's *t*-test).

Immunoblotting

IgG-class AMA to a 21.5 kD mosquito antigen, a major salivary antigen of *A. communis* mosquitoes, was measured by using whole-body extracts of *A. communis* mosquitoes in immunoblotting as previously described [12]. In brief, the mosquito extracts were run in sodium-dodecyl sulphate polyacrylamide gels at constant voltage (Mini Protean II Cell, Bio-Rad, Richmond, CA) and transferred to nitrocellulose membrane (Trans-Blot, Bio-Rad). First the serum samples (diluted 1/50) taken before and after 10-day mosquito exposure were added, followed by goat anti-human IgG peroxidase-conjugate (Bio-Rad) after which the strips were developed. The AMA to 21.5 kD antigen were read and scored as negative (–) or positive (+, ++). The samples of 21 volunteers were analysed by immunoblotting only because these subjects were exposed very early in the mosquito season, and they had received no mosquito bites during the previous 6–7 months.

Results

Cutaneous reactivity to mosquito bites

Twenty-five of the volunteers reacted to mosquito bites with immediate and/or delayed (Fig. 1) cutaneous lesions, but two subjects were non-responsive. The mean diameter of skin lesion and degree of pruritus decreased from 15 min to 24 hr after the bites (Fig. 2). Table 1 shows the different patterns of skin reactivity in the 21 volunteers exposed to mosquitoes at the onset of the season. Two of these volunteers were non-responsive, two had only immediate (diameters 9 mm and 15 mm) and two only delayed lesions (diameters 5 mm and 25 mm). The remaining 15 subjects had various combinations of immediate and delayed reactions; three had strong immediate and delayed reactions, one subject had a weak immediate and strong delayed, five had strong immediate and weak delayed, and six subjects had both weak immediate and delayed reactions.

According to their personal history and prick test results, eight of the volunteers were atopics but the immediate bite lesions (mean diameter 8.3 mm) and pruritus (mean score 43) were similar to those of 19 non-atopic volunteers (mean lesion diameter 7.3 mm, pruritus score 41).

Effect of cetirizine

Cetirizine significantly decreased the weal response at 15 min ($P<0.01$), but had no significant effect on bite lesions at 60 min (Fig. 3). The effect on pruritus was significant ($P<0.01$) at 15 min (Fig. 4) and also when the 15 min and



Fig. 1. Immediate weal (top) and a delayed papule (below) from the bite of *Aedes communis* mosquito.

60 min results were combined and analysed separately ($P < 0.01$). Many of the delayed skin lesions were small and pruritus scores low or negative already in the baseline period. Due to this only those subjects were included in the statistical analysis who at 12 hr and 24 hr had skin lesions over 2 mm in diameter and in whom the pruritus scores were at least 6. Cetirizine had no significant effect over placebo on the skin lesions at 12 hr ($n = 10$; $P = 0.49$) or at 24 hr ($n = 12$; $P = 0.08$). The effect of pruritus was also non-significant both at 12 hr ($n = 10$; $P = 0.46$) and at 24 hr ($n = 9$; $P = 0.77$).

Two subjects in the cetirizine-treated group and one in the placebo group experienced sedation, but no other side-effects were observed during the study.

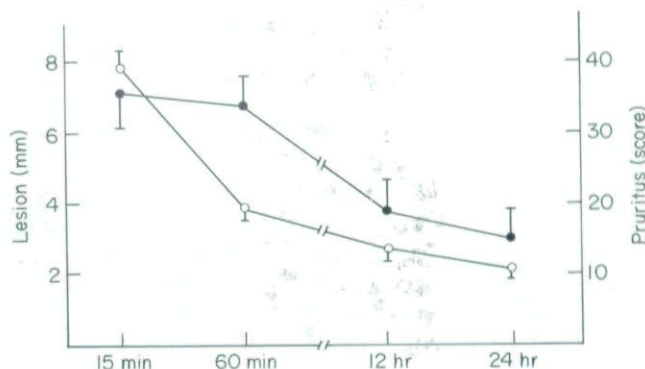


Fig. 2. Cutaneous reactivity to *Aedes communis* mosquito bites in 27 normal adult subjects. The size of the skin lesion (mean $\pm$ s.e.m.; ●) and the degree of pruritus (mean $\pm$ s.e.m.; ○) decreases similarly after the bites.

IgG anti-mosquito antibodies to 21.5 kD antigen

Four of the 21 volunteers (19%) had AMA before the mosquito exposure. After the 10-day exposure immunoblotting showed a positive result in 10 subjects (48%) (Fig. 5). In three of the four subjects with positive pre-exposure samples the AMA remained at the same level (+ + in one, + in two subjects), whereas in the remaining volunteer the 21.5 kD band seemed to be more intense after mosquito exposure.

The IgG-AMA occurred in the post-exposure serum samples of five (83%) of the six volunteers with a strong delayed and in five (33%) of the 15 volunteers with weak or no delayed bite reactions. The frequency of AMA was 6/9 (67%) in subjects with strong immediate and 4/11 (36%) in those with weak or no immediate reactions. No AMA were detected in the two non-responsive volunteers. The occurrence of AMA showed no correlation with the total number of mosquito bites; the mean number of bites was 49 (range 14–67 bites) in the AMA-positive and 45 (range 21–63 bites) in the AMA-negative group.

Discussion

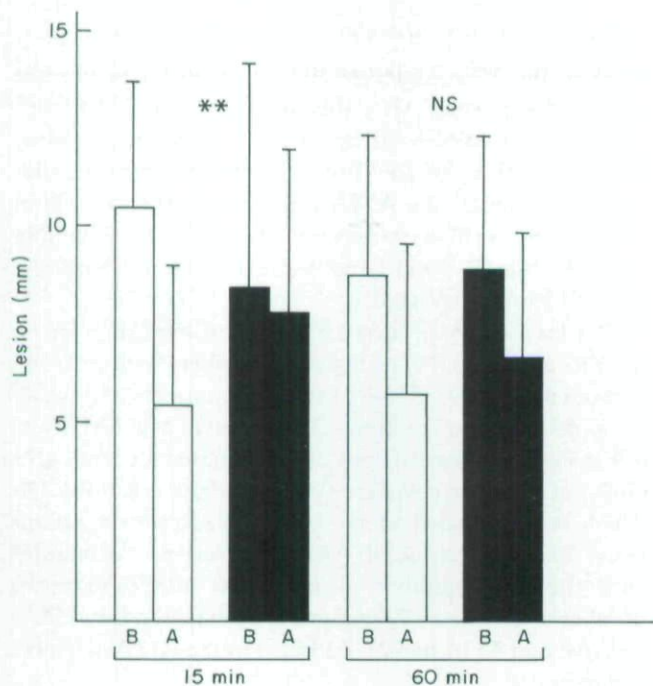
The patterns of mosquito-bite reactions among the present volunteers ranged from non-reactivity to various combinations of immediate wealing and delayed bite-papules. This result, obtained at the onset of mosquito-season to *Aedes communis* bites, shows that normal subjects exhibit different stages of cutaneous sensitivity to a mosquito species they have been exposed for years. It is impossible to know how many bites the volunteers had received during their life. Exposure to mosquitoes is high in rural areas surrounded by forests as well as northern parts of Finland [13,14]. Two of the present volunteers were non-reactive, and they both had a previous history

Table 1. Skin reactivity to *Aedes communis* bites in 21 normal adult subjects at the onset of mosquito season

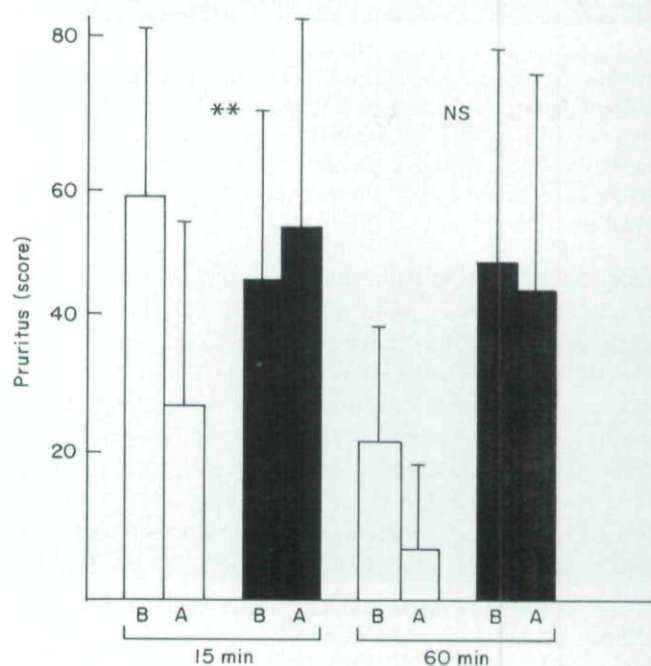
Stage of reactivity*	No. of subjects	Mean diameter of immediate (Imm)	Mean diameter of delayed (Del)
		15 min lesion (mm)	24 h lesion (mm)
Stage 2 (Imm-, Del+)	2	0	15
Stage 3 (Imm+, Del+)	15	8.9 ± 4.7†	3.7 ± 2.0†
Stage 4 (Imm+, Del-)	2	12	0
Stage 5 (Imm-, Del-)	2	0	0

* According to [3]

† Mean ± s.d.

**Fig. 3.** The size of the 15 min and 60 min skin lesions (mean ± s.d.) from the *Aedes communis* mosquito bites in the cetirizine- and placebo-treated volunteer groups before (B) and after (A) drug treatment. The effect of cetirizine is significant on 15 min lesions ($P < 0.01$) but not significant on 60 min lesions ($P < 0.51$; NS). □, cetirizine. ■, placebo.

of extensive mosquito exposure during which their bite reactions had faded gradually. This suggests that both these subjects had been desensitized by the excessive bites they had received. In agreement with this view, experimental mosquito-bite studies have presented data allowing the classification of man's reaction to mosquito bites in five different stages. After the initial non-reactive stage

**Fig. 4.** The degree of pruritus (mean score ± s.d.) 15 min and 60 min after *Aedes communis* mosquito bites in the cetirizine- and placebo-treated volunteer groups before (B) and after (A) drug treatment. The effect of cetirizine is significant on pruritus score at 15 min ($P < 0.01$) but not significant at 60 min ($P = 0.42$; NS). □, cetirizine. ■, placebo.

(stage 1), subsequent bites cause a delayed cutaneous reaction (stage 2), then after several months immediate wealing develops (stage 3); thereafter the delayed bite-papule disappears (stage 4), and finally the immediate reaction (stage 5) [3].

Mosquito-bite reactions are generally considered to be immunological in nature [7]. Mosquitoes inject saliva into the skin when feeding, and this seems to sensitize the



Fig. 5. Development of IgG anti-mosquito antibodies after 40–60 *Aedes communis* mosquito bites in rabbit and man. Whole-body *A. communis* extract was used as antigen in immunoblot studies. IgG antibodies against 21.5 kD protein (arrow) are seen both in the post-exposure serum (lane 2) from the rabbit (R) and human subject (H). Pre-exposure sample (lane 1) from the rabbit is totally negative for this antibody but pre-exposure serum from human subject shows a very faint line at the 21.5 kD location.

recipient. If the salivary ducts are cut, no cutaneous reactions are observed in a sensitized subject [15]. The antigens in mosquito saliva and the mechanisms causing the various bite reactions have remained for the most part obscure. The weal reaction seems to be histamine-mediated, but there are very few reports describing IgE-class AMA in man [11,16]. We have developed an immunoblot method for measuring IgG-class AMA, and have shown that, in rabbits, 40–60 *Aedes communis* bites cause antibody response directed against a 21.5 kD salivary antigen [12]. In man, similar IgG-class AMA to the 21.5 kD antigen can be detected in the sera of children and adults, but not in infants never exposed to mosquitoes [12]. A few other studies have detected the occurrence of IgG-class AMA in man by means of ELISA [9], immunohistochemistry [10], or immunoblot techniques [11,17], but to our knowledge there are no previous studies measuring these antibodies before and after exposure to mosquitoes. In the present study, 19% of the volunteers had these antibodies before the mosquito season but after the 10-day exposure to a mean of about 50 bites, the frequency was 48%. This suggests that an IgG-antibody response also occurs in man after only a few mosquito bites.

In the present study the frequency of IgG-AMA was clearly higher in those subjects who exhibited strong cutaneous bite-reactions. On the other hand, several subjects with positive reactions were negative for AMA suggesting that these antibodies may not be pathogeneti-

cally important. However, our immunoblot method using whole-body mosquito extract as antigen may be too insensitive to detect low AMA levels in man. In a previous study we showed that rabbits exposed to about 40 *A. communis* mosquito bites developed skin reactivity at the same time as the IgG-class AMA to 21.5 kD antigen appeared in the serum [12]. Our recent finding that the subclass of IgG-AMA is mainly IgG4 in man (Brummer-Korvenkontio *et al.*, manuscript in preparation) substantiates the view that the IgG-class AMA response could be similar to that observed during hyposensitization treatment [18].

The weal reaction to mosquito bites is commonly thought to be histamine-mediated, but evidence for this is scant [7]. Previously, Coulie *et al.* [19] showed that cetirizine reduced pruritus but had no significant effect on skin lesions occurring after mosquito bites in laboratory conditions. In the present study, cetirizine significantly reduced the weal response and pruritus after natural mosquito bites supporting the contention that immediate mosquito-bite symptoms derive from the action of histamine. Cetirizine is a potent, non-sedating antihistamine which in addition to its anti-H1 action also markedly inhibits cutaneous late-phase reactions [20], a mechanism that may also be involved in mosquito-bite reactions. In the present study cetirizine had no significant effect on the delayed 12 hr or 24 hr bite lesions, but the small number of individuals in the present series does not allow definite conclusions on this point.

The present finding that cetirizine effectively decreased wealing and pruritus in normal subjects after mosquito bites is important also for clinical practice. When mosquito bites cannot be avoided the prophylactic use of cetirizine can be helpful. In areas of heavy mosquito infestation, clothing and repellents do not provide total protection from the bites; moreover, continuous use of repellents containing high concentrations of diethyltoluamide may be dangerous, especially for children [21,22]. Further investigation is needed to clarify whether cetirizine could in addition to normal subjects also help people exhibiting abnormal reactions to mosquito bites and to compare its efficacy with that of other antihistamines.

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