

Effect of cetirizine on the inflammatory cells in mosquito bites

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Summary

Background Mosquito bites frequently cause wealing and delayed papules which appear within a few hours after the bites and may persist for several days. Cetirizine is an effective drug against mosquito bites by decreasing wealing and also the size and pruritus of the delayed bite papules.

Objectives To characterize inflammatory cells in the delayed mosquito-bite lesions, and to study the effect of cetirizine on the inflammatory cell response.

Methods Twenty-six mosquito-bite sensitive subjects received cetirizine 20 mg (14 subjects) or placebo (12 subjects) in a double-blind fashion. *Aedes aegypti*-bites were given on a forearm and serial punch biopsies were taken at 2-, 6- and 24 h after the bite exposure. Eosinophils, neutrophils, mast cells, mononuclear cells and T- helper (CD4⁺) and suppressor (CD8⁺) lymphocytes were counted from dermal infiltrates.

Results Eosinophils and neutrophils were found already in 2-h bite lesions. Moreover, the number of mononuclear and CD4⁺ cells increased significantly ($P < 0.01$) from 2- to 24-h bite lesions. Unexpectedly, the overall numbers of eosinophils ($P < 0.05$), mononuclear cells ($P < 0.01$) and CD4⁺ cells ($P < 0.01$) were significantly higher in the cetirizine-treated subjects compared with the placebo-treated subjects.

Conclusions The results suggest that the inflammatory cell response in the delayed mosquito-bite lesions is similar to that occurring in allergic late-phase responses, i.e. an early influx of eosinophils, neutrophils and subsequent accumulation of CD4⁺ lymphocytes. The reason for the high numbers of eosinophils and CD4⁺ cells in the cetirizine-treated subjects is not known.

Keywords: *Aedes* mosquitoes, delayed bite reactions, eosinophils, lymphocytes, cetirizine treatment

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Introduction

Mosquitoes frequently cause harmful bite reactions such as immediate weals and delayed bite papules. A humoral immune response following mosquito bites seems to be a ubiquitous phenomenon because both children and adults have immunoglobulin E (IgE) and IgG4 antibodies against mosquito saliva antigens [1,2]. Mosquito-bite wealing has

been shown in passive transfer studies to be mediated by antisaliva IgE antibodies [3]. Inversely, the mechanism of the delayed bite lesions which appear 2–6 h after the bites and may persist for several days is not known.

Eosinophils occur commonly in delayed insect bite lesions and have been reported also in biopsies taken from mosquito bites [4,5]. These findings suggest that the delayed mosquito-bite lesions could be cutaneous late-phase reactions initiated by mast-cell-derived mediators and resulting in the influx of eosinophils [6]. Cetirizine, a potent H₁-blocking antihistamine, has been shown to act also on

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eosinophils *in vivo* [7,8]. A double-blind, placebo-controlled study performed in mosquito-bite-sensitive subjects showed that cetirizine 10 mg is effective against mosquito-bite wealing and, interestingly, also against the delayed bite papules [9].

In the present study, we examined inflammatory cells, especially eosinophils and lymphocytes, in the delayed mosquito-bite lesions. Twenty-six mosquito-sensitive subjects were challenged and serial biopsies were taken from the lesions 2, 6 and 24 h after the bites. To examine the effect of cetirizine on the inflammatory cell response, the subjects were given either cetirizine 20 mg or placebo during the study.

Subjects

Twenty-six subjects (24 females, two males; mean age 41, range 19–65 years) volunteered for the study. All had a history of pruritic weals and delayed bite papules from mosquito bites in the field, and 24 subjects had been referred to the Department of Dermatology at the Tampere University Hospital. Their mosquito sensitivity was confirmed by bite challenge with *Aedes aegypti* mosquitoes. All 26 subjects gave their informed consent, and the biopsy study was approved by the Ethical Committee of the hospital. The study was performed outside the mosquito season and the subjects were not allowed to take antihistamines 2 weeks (astemizole 6 weeks) before the study.

Drug administration and mosquito challenge

The subjects were given in a double-blind fashion either cetirizine 20 mg (14 subjects) or placebo tablets (12 subjects) once a day (at 08.00 h) for 4 days.

The mosquito challenge was performed on day 3 between 13.00 h and 16.00 h. Adult *Aedes aegypti* mosquitoes were from the Laboratory of Protozoology, Institute of Tropical Medicine, Antwerp, Belgium. Three mosquitoes were put in a special cage which was placed on the forearm. The mosquitoes were allowed to bite and feed to repletion as described previously [3]. The bites were marked, the diameters measured, and the biopsies were taken at 2, 6 and 24 h.

Skin biopsies and cell counting

A punch biopsy (diameter 4 mm) was taken after injection of local anaesthetic (1% lidocaine without epinephrine). The biopsy specimens were divided into two pieces; the one was fixed in formalin and embedded in paraffin and the other was snap frozen in liquid nitrogen, embedded in mounting medium (Tissue Tek OCT, Miles Inc. Elkhart, IN, USA) and then stored at -70°C .

Eosinophils, neutrophils and mononuclear cells were counted from paraffin sections stained with haematoxylin and eosin. Mast cells were counted from toluidine blue stained sections. Slides were counted blind with a light microscope using an eyepiece graticule and $600\times$ magnification. The counting was performed from at least three 0.027 mm^2 fields on the cell infiltrates in the papillary and middermis.

T lymphocyte subsets (CD4 and CD8 positive cells) were identified in acetone- and chloroform- fixed $5\text{ }\mu\text{m}$ cryostat sections using monoclonal antibodies, anti-Leu-3a + 3b (CD4) and anti-Leu-2a (CD8) obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA, USA). Bound primary antibodies were visualized with the streptavidin-biotin peroxidase technique (Vectastain Elite Kit, Vector Laboratories, Burlingame, CA, USA) according to manufacturer's instructions. Diaminobenzidine was used as chromogen. Endogenous peroxidase activity was blocked after the secondary antibody with 0.3% H_2O_2 in methanol. CD4⁺ and CD8⁺ cell counting was similar to that performed in paraffin sections.

Statistics

The data were analysed on an intention-to-treat basis, so all randomized subjects (14 on cetirizine and 12 on placebo) were included in the analysis. All tests were two-tailed and performed at the 5% level of significance. As the distribution of inflammatory cell counts were not normally distributed, and the variances between the cetirizine and placebo-treated groups were not similar within and between various time points (non-homoscedasticity of the variance/covariance matrix), the data were analysed by means of non-parametric tests. Treatment differences at each time points (2, 6 and 24 h) and the overall treatment effect were analysed by means of the Mann–Witney *U*-test; the same test was used to analyse the size of the mosquito-bite reactions. The overall time effect was analysed with the Kruskal–Wallis test. Data management and statistics were performed with SAS software (SAS Institute Inc, USA).

Results

Treatment groups and bite reactions

All 14 subjects in the cetirizine and 12 patients in the placebo-group completed the study. The two groups were similar in age and in personal history of atopy. In the cetirizine group the mean age was 43.4 years (range 19–65 years) and in the placebo-group 37.3 years (range 22–63 years). Two patients in the cetirizine and one in the placebo-group had skin-prick test proven allergic rhinitis but none had asthma nor atopic dermatitis.

The pretreatment *A. aegypti*-bite exposure showed larger 15 min weals in the cetirizine than in the placebo-group (mean diameters 8.2 ± 2.9 and 5.2 ± 1.7 mm; $P = 0.004$) whereas the 24-h bite reactions were similar (mean diameters 3.8 ± 3.5 and 3.9 ± 2.5 mm; $P = 0.68$). During the study the mean diameters of 15 min and 24-h bite lesions were somewhat lower in the cetirizine group. However, no significant differences were observed between the cetirizine and placebo-treated groups either in the diameters of 15 min weals (mean 7.0 ± 3.0 and 5.6 ± 1.9 mm; $P = 0.53$) or 24-h bite lesions (mean 3.5 ± 2.3 and 4.8 ± 2.5 mm; $P = 0.31$).

Biopsy specimens

A total of 73 biopsy specimens was taken for the study. All three, 2-, 6- and 24-h, biopsy specimens were available from 23 subjects (12 received cetirizine, 11 placebo). In two subjects the mosquitoes did not bite adequately; only a 24-h biopsy specimen was obtained. One subject failed to attend the 6-h visit, but the 2- and 24-h biopsies were taken according to the protocol. Sixty-five of the 73 specimens were of adequate quality for eosinophil and neutrophil counting. Seventy-two of the 73 specimens were evaluable for mononuclear cells, and 71 for CD4⁺ and 60 for CD8⁺ suppressor cells.

Eosinophils

The biopsy specimens from the placebo-treated subjects

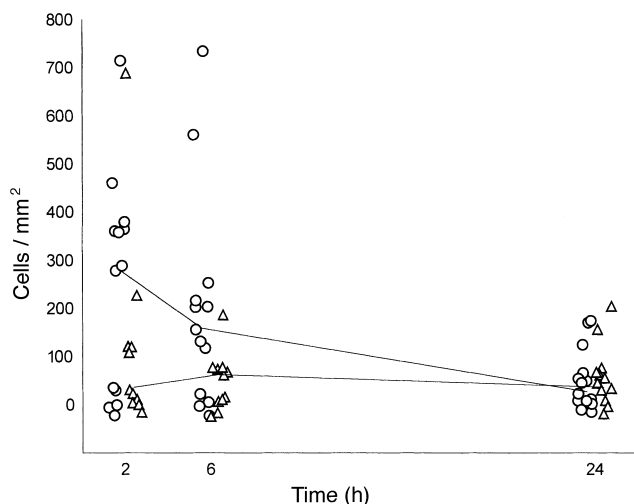


Fig. 1. Individual and median numbers of eosinophils (cells/mm²) in the 2-, 6- and 24-h mosquito-bite specimens. Thirteen subjects received cetirizine (O) and 11 received placebo (Δ). Overall, the median number of eosinophils is significantly ($P = 0.04$) higher in the cetirizine than in the placebo group. Treatment difference is significant ($P < 0.05$) at 6 h but not at 2 and 24 h.

showed cell infiltrates with eosinophils already at 2 h. Individual variation was large (Fig. 1); a few subjects had high eosinophil counts (maximum 678 cells/mm²) in the infiltrates whereas no eosinophils were detected in four of the 11 representative specimens. Eosinophils were mainly seen in the upper dermis around the vessels (Fig. 4) but in some specimens cells were also found between collagen fibres far from the vessels. Extracellular granules were also observed in many specimens.

Overall, the median number of eosinophils was significantly higher in the cetirizine group as compared with the placebo group ($P = 0.038$; Fig. 1). Treatment difference was significant only at 6 h ($P = 0.022$), and no time effect ($P = 0.12$) was observed.

Neutrophils

In the placebo-treated subjects the median numbers of neutrophils were similar in the 2-, 6- and 24-h biopsy specimens (Fig. 2). As with eosinophils, the individual variation was large, one 2-h specimen showing 533 cells/mm². In contrast, four of the 11 representative specimens taken at 2 h did not show any neutrophils.

The median numbers of neutrophils were comparable in both treatment groups at 2, 6 and 24 h ($P = 0.37$), and no significant time effect ($P = 0.056$) was noticed (Fig. 2).

Mast cells

The median numbers of mast cells were low on placebo and cetirizine treated groups, and remained nearly constant in

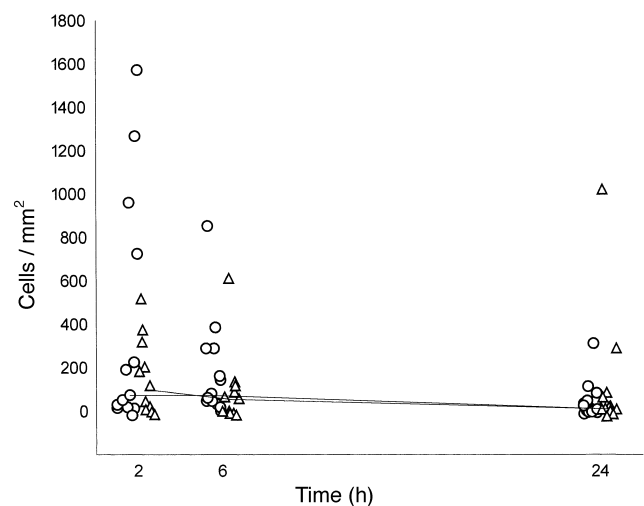


Fig. 2. Individual and median numbers of neutrophils (cells/mm²) in the 2-, 6- and 24-h mosquito-bite specimens. The subjects and symbols are the same as in Fig. 1. The cetirizine and placebo groups show similar numbers of neutrophils.

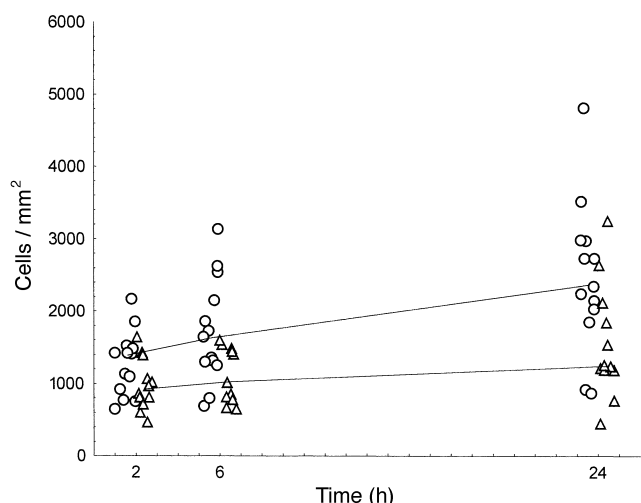


Fig. 3. Individual and median numbers of mononuclear cells (cells/mm²) in the 2-, 6- and 24-h mosquito-bite specimens. The subjects and symbols are the same as in Fig. 1. Overall, the median number on cetirizine treatment was significantly higher ($P = 0.002$) as compared with the median in the placebo-treated subjects. The differences were also significant between the cetirizine and placebo groups in the 6-h ($P = 0.049$) and 24-h ($P = 0.02$) specimens.

the 2-, 6- and 24-h specimens (Table 1). Thus, no significant difference for time or treatment was found.

Mononuclear cells

In contrast to eosinophils, neutrophils and mast cells, the

number of mononuclear cells increased significantly with time ($P = 0.002$; Fig. 3). Overall, the median number in cetirizine-treated subjects was significantly higher ($P = 0.002$) than the median in the placebo-treated subjects. At 6 and 24 h, the median was significantly higher ($P = 0.049$ and $P = 0.024$) in the cetirizine group than in the placebo group (Fig. 3). The highest individual numbers of mononuclear cells in the 24-h biopsy specimens were 4815 cells/mm² in the cetirizine and 3241 cells/mm² in the placebo-treated group.

CD4⁺ and CD8⁺ cells

Like mononuclear cells, CD4⁺ cells increased significantly ($P = 0.0001$) with time (Table 1). Overall, the cetirizine-treated subjects had significantly higher ($P = 0.013$) numbers of CD4⁺ cells than the placebo-treated subjects. The difference was significant in the 6-h ($P = 0.032$) and 24-h ($P = 0.050$) specimens. High numbers of CD4⁺ cells in a 24-hour biopsy specimen are seen in Fig. 5.

The median numbers of CD8⁺ cells were lower than those of CD4⁺ cells (Table 1). In contrast to CD4⁺ cells, no time effect was observed for CD8⁺ cells. However, a significant ($P = 0.001$) overall treatment effect was noticed, and the median number of CD8⁺ cells was significantly higher ($P = 0.036$) in the 24-h biopsies in the cetirizine-treated subjects as compared with placebo-treated subjects (Table 1).

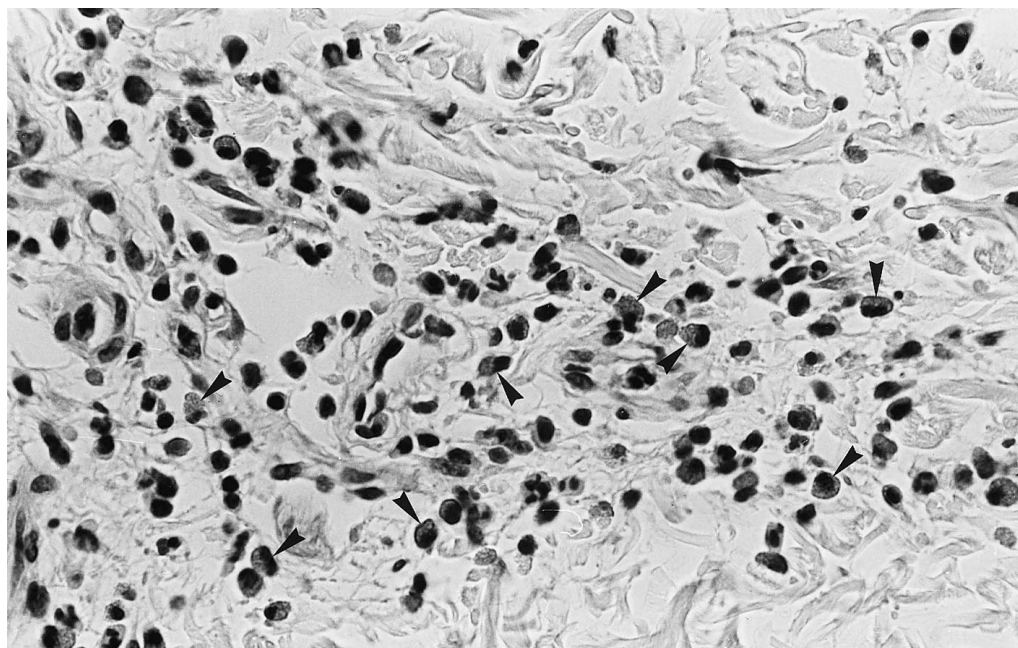


Fig. 4. Eosinophils (→) are abundant in the perivascular cell infiltrate of the 2-h mosquito-bite specimen taken from a patient (E.S) who received cetirizine. Haematoxylin-eosin staining (original magnification 132×).

Table 1. The numbers of mast cells, T-helper and suppressor cells in the 2-, 6- and 24-h mosquito-bite lesions

Cells	Treatment groups	Biopsy specimens			P-value	
		2 h	6 h	24 h	Time-effect	Treatment effect
Mast cells	Cetirizine	29 (7, 51)	37 (15, 64)	19 (15, 29)	NS	NS
	Placebo	37 (22, 46)	37 (29, 44)	22 (22, 29)		
	P-value	NS	NS	NS		
T-helper cells	Cetirizine	307 (270, 556)	567 (407, 915)	1759 (1333, 2185)	0.0001	0.013
	Placebo	213 (139, 306)	285 (167, 481)	710 (385, 1344)		
	P-value	NS	0.032	0.050		
T-suppressor cells	Cetirizine	241 (159, 370)	285 (130, 481)	630 (381, 870)	NS	0.001
	Placebo	148 (43, 278)	137 (0, 196)	174 (50, 463)		
	P-value	NS	NS	0.036		

* Median numbers of the cells/mm², the first and third quartiles in parentheses. NS. Not significant.

Discussion

In the present study, we examined cutaneous inflammatory cell response in cetirizine- and placebo-treated mosquito-bite-sensitive subjects. The bite challenges were performed with *Aedes aegypti* mosquitoes, and serial biopsies were taken from the lesions at 2, 6 and 24 h after the bites. All placebo-treated subjects showed wealing 15 min after the bites, and most specimens taken from 2-h lesions showed cell infiltrates with eosinophils and neutrophils. Thereafter, these cells were found in the 6- and 24-h bite lesions and no significant change occurred in their numbers. An important finding was a significant increase of mononuclear cells from 2- to 24-h bite lesions. Interestingly, CD4⁺ but not CD8⁺ cells showed a parallel significant increase. These results obviously indicate that the inflammatory cell response in mosquito-bite lesions is characterized by an early influx of eosinophils and neutrophils with a subsequent increase in the numbers of infiltrating CD4⁺ cells.

In experimental cutaneous late-phase reactions produced by ragweed allergen and examined in suction blister chambers eosinophils and neutrophils have been detected from 4 h after the antigen challenge [8], i.e. somewhat later than in the present mosquito-bite lesions. However, Leiferman *et al.* [10] observed extensive deposition of eosinophil major basic protein and neutrophil elastase in the dermis as early as 1–3 h after the ragweed challenge. Therefore, their results in cutaneous late-phase reactions are in agreement with the present findings of an early influx of eosinophils and neutrophils in the delayed mosquito-bite lesions. We also found CD4⁺ and CD8⁺ cells already in 2-h bite lesions. Thereafter, the numbers of CD4⁺ cells increased significantly and were highest in 24-hour lesions. CD8⁺ cells showed no such increase, and they were about four times

lower in number than CD4⁺ cells in 24-h lesions. Overall, the inflammatory cell response observed in the present delayed mosquito-bite lesions fits well to the findings occurring after intradermal injection of respiratory allergens [10,11]. Mosquito-bite wealing is mediated by IgE-antislava antibodies [3]. It seems therefore that the delayed mosquito-bite reaction is a 'natural' model for studying allergic late-phase inflammatory cell response in the skin.

An unexpected finding of the present mosquito-bite biopsy study was the effect of cetirizine on the inflammatory cell response. Cetirizine is known to inhibit eosinophil migration and decrease cutaneous late-phase reactions *in vivo* [7,8,12]. Moreover, we have previously shown in mosquito-bite-sensitive subjects that cetirizine 10 mg decreases the size of the 12- and 24-h mosquito-bite lesions and diminishes significantly the accompanying pruritus [9]. Therefore, we expected to find decreased numbers of eosinophils in the bite lesions of the present cetirizine-treated subjects. The results showed, however, that the overall number of eosinophils was significantly higher in the cetirizine-treated than in the placebo-treated subjects. We found, however, no significant differences in the size of the 15 min and 24-h bite lesions of the two treatment groups. The reason for this could be that the subjects were bitten by *A. aegypti* laboratory mosquitoes which caused rather small immediate and delayed bite reactions in the placebo-treated subjects. In contrast, in our previous cetirizine treatment study the subjects were bitten in the field by *A. communis* mosquitoes which caused much bigger bite reactions [9].

The present treatment groups did not differ in age or occurrence of atopy but one confounding factor could be that cetirizine-treated subjects showed before the study bigger mosquito-bite wheals than placebo-treated subjects. However, the size of the delayed bite lesions were similar in

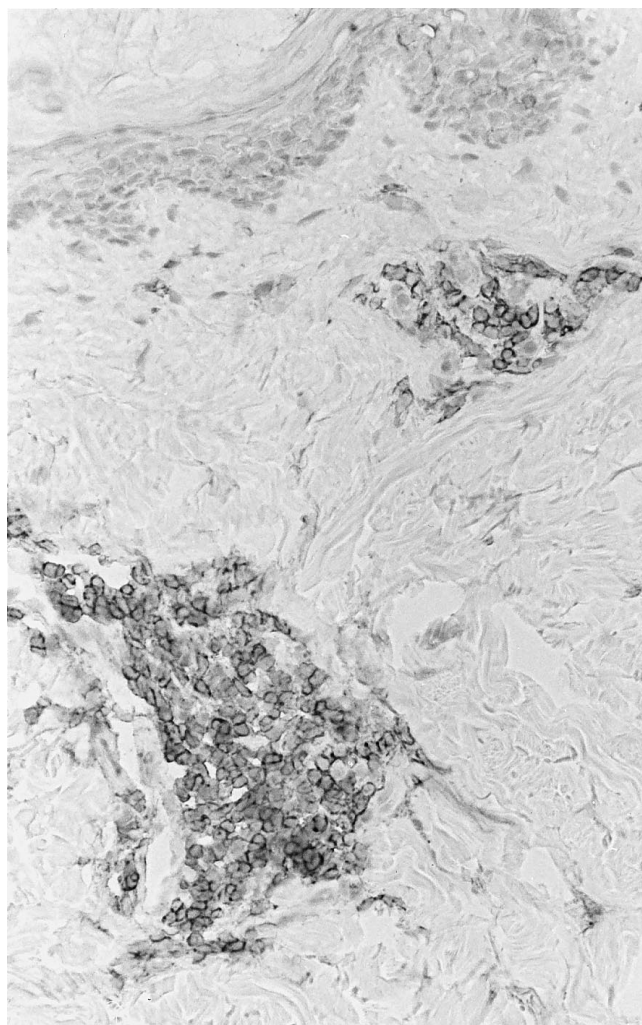


Fig. 5. Perivascular cell infiltrates showing CD4⁺ lymphocytes in the 24-h mosquito-bite specimen taken from a patient (E.S) who received cetirizine. Peroxidase staining (original magnification 66×).

both groups. This suggests that the slight pretreatment difference in the immediate mosquito-bite reactivity is not the reason for the significantly increased eosinophil and CD4⁺ cell infiltration in the delayed bite reactions of the cetirizine-treated subjects. One possible explanation for the increased eosinophil counts could be that cetirizine blocks the migration of these cells by modulating their adhesion molecule expression and consequently, these cells become inactive 'bystanders' in the skin [13–15]. Cetirizine has been reported to reduce eosinophil vacuolization in the cutaneous late-phase reactions [16]. We observed degranulated eosinophils in several biopsy specimens taken from placebo- but also from cetirizine-treated subjects but unfortunately, we could not measure eosinophil degranulation in these specimens. Therefore, it is impossible to say whether

the increased amounts of eosinophils in the inflammatory infiltrates in cetirizine-treated subjects are inactive 'bystanders' whose survival is prolonged, e.g. by autocrine excretion of cytokines such as IL-3 or IL-5 [17]. Similar mechanisms could be the reason for the increased numbers of mononuclear and CD4⁺ cells in the cetirizine-treated subjects as compared with the placebo-treated subjects. In general, the present results are in line with the previous observations that cetirizine decreases eosinophils in the allergic late-phase reactions [7,8,12] because we observed clearly higher numbers of eosinophils in the 2-h mosquito-bite lesions compared with the 24-h lesions.

In conclusion, the present study documented an early influx of eosinophils, neutrophils and mononuclear cells in the delayed mosquito-bite lesions. The numbers of mononuclear and CD4⁺ cells increased significantly from 2- to 24-h lesions whereas no time effect was observed for other cells. Moreover, cetirizine-treated subjects had significantly higher overall number of eosinophils and mononuclear cells than placebo-treated subjects, a finding which suggests that the effect of cetirizine on delayed mosquito-bite lesions is not due to inhibition of cellular recruitment.

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