

Cellular inflammatory responses and mediator release during early developing late-phase allergic cutaneous inflammatory responses: Effects of cetirizine

Paul C. Atkins, MD, Burton Zweiman, MD, Anne Moskovitz, Carolyn von Allmen, and Michael Ciliberti, MD Philadelphia, Pa.

Background: Events in developing cutaneous late-phase allergic reactions can be characterized by a combination of skin chamber and biopsy approaches. In some previous studies, cetirizine reportedly inhibited mediator release and/or inflammatory cell responses in late-phase reactions.

Objective: This study was carried out to determine the effects of cetirizine on early late-phase reactions by using skin chamber and skin biopsy specimens.

Methods: Skin chamber responses during a 6-hour challenge with pollen antigens were assessed in 15 sensitive subjects during randomized, crossover treatment with cetirizine (20 mg/day) or placebo for 7-day periods with measurements of humoral and cellular components. Biopsy specimens of the underlying dermis were obtained.

Results: During cetirizine treatment, there was significant ($p < 0.01$) inhibition of immediate wheal and flare reactions to pollen antigens (34, 46%), codeine (41, 65%), and histamine (38, 68%). However, gross late-phase reactions at 6 hours were unaffected. During both cetirizine and placebo treatment, there was significantly greater accumulation at antigen sites in: (1) skin chamber levels of histamine, total cells, lactoferrin, and eosinophil cationic protein; (2) eosinophils (total and activated) on appended cover glasses; (3) deposition of lactoferrin and eosinophil cationic protein in the underlying dermis. However, these responses were not significantly different during cetirizine treatment compared with placebo treatment periods.

Conclusion: A persistent pattern of inflammatory cell accumulation with release of granule proteins during early late-phase reactions was unaffected by cetirizine treatment. (J Allergy Clin Immunol 1997;99:806-11.)

Key words: Late-phase response, cutaneous allergic response, neutrophils, eosinophils, lactoferrin, ECP, cetirizine

Late-phase allergic cutaneous and bronchial responses generally peak 4 to 12 hours after antigen exposure.¹ The pathogenic events during the first 4 to 8

Abbreviations used

ECP:	Eosinophil cationic protein
HPF:	High power field
LPR:	Late-phase reaction
PBS:	Phosphate-buffered saline
PNU:	Protein nitrogen units

hours of challenge (called "early" late-phase reactions) may determine the manifestations during the succeeding 24 hours. Therefore they warrant particular scrutiny.

Using a skin chamber model, we have reported a number of events during continuous antigen challenge of reactive human beings including: (1) early release of histamine, tryptase, and prostaglandin D_2^2 ; (2) the later release of histamine without other mast cell mediators (likely from basophils²); and (3) a later appearance of inflammatory cells and their granular components.^{3,4} By using a combination of skin chamber approaches, cover glasses appended to the challenge sites at the base of the skin chamber, and biopsy specimens of the underlying dermis, we have been able to assess mechanisms involved in the effects of therapeutic agents used in allergic diseases.⁵

We have recently applied these approaches to investigate effects of the nonsedating antihistamine cetirizine, an active metabolite of hydroxyzine. Several studies have reported varying degrees of antiinflammatory effects of cetirizine in allergic reactions with the use of skin windows, skin chambers, and ocular conjunctival challenges.⁶⁻¹⁴ In some studies cetirizine inhibited skin, pulmonary, and ocular late-phase responses (LPRs).^{8,12,14} However, most previous studies used relatively small numbers of subjects, and the effects of cetirizine were not consistent. Therefore we have used a combination of our skin chamber model, cover glasses appended to the chamber bases, and biopsy specimens of the underlying dermis to more precisely assess the effects of cetirizine on mediator release, inflammatory cell accumulation, and activation during developing "early" LPRs in the first 6 hours of antigen challenge.

From the Allergy and Immunology Division, Department of Medicine, University of Pennsylvania School of Medicine.

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Reprint requests: Burton Zweiman, MD, Allergy and Immunology Division, Department of Medicine, University of Pennsylvania School of Medicine, 512 Johnson Pavilion, 36th and Hamilton Walk, Philadelphia, PA 19104-6057.

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METHODS

Subjects and protocol

Fifteen subjects, who had experienced both immediate responses and LPRs after intradermal injections of 10 and 100 protein nitrogen units (PNU)/ml ragweed or grass extract, were selected. All studies were performed outside the grass and ragweed pollen seasons. After informed consent was obtained, subjects received cetirizine or placebo, 20 mg/day, for 7 days with a 14-day drug-free interval, in a random crossover design. On the following days, each subject underwent the procedures as listed below.

Days 6 and 27

Determination of cetirizine levels. Blood was obtained before ingestion of cetirizine for determination of plasma cetirizine levels. Plasma cetirizine was assayed by Pfizer Pharmaceuticals with high-performance liquid chromatography; the lower level of sensitivity was 5 ng/ml.

Wheal and flare responses. Wheal and flare responses were measured at 20 minutes, and erythema and induration were measured at 6 hours after intradermal injection of 0.03 ml of phosphate-buffered saline (PBS), histamine (1 mg/ml), codeine (1 mg/ml), and antigen (serial 10-fold dilutions from 100 to 0.0001 PNU/ml).

Days 7 and 28

Skin chamber studies. Skin chamber studies were performed as previously described.²⁻⁴ In brief, four blisters were induced on the volar surface of the forearms by gentle heat and suction. The epidermal blister roofs were removed from the blister bases at the dermal-epidermal junction, and collection chambers were appended. After two 5-minute irrigations of the blister bases with buffer diluent (PBS), 0.3 ml of grass or ragweed pollen antigens (Greer Labs., Lenoir, N.C.), diluted to 100 PNU/ml in PBS containing heparin (10 U/ml), was placed in chambers at two sites. PBS containing heparin 10 U/ml was placed in chambers at the other two sites. After 1 hour, the fluids were removed, and the fluids from duplicate chambers were pooled. They were then assayed for histamine levels by ELISA (Immunotech), sensitive to 0.1 ng/ml. Fresh antigen and PBS solutions containing heparin, 10 U/ml, were placed in the relevant chambers and allowed to remain for an additional 5 hours. Fluids from challenge chambers were removed, the duplicate fluids were pooled, and total cell counts were obtained. The two pooled fluids were then centrifuged, and supernatants were analyzed for levels of histamine, eosinophil cationic protein (ECP) (radioimmunoassay, Pharmacia, sensitive to 2 ng/ml), and lactoferrin (ELISA, sensitive to 2 µg/ml). The cell pellets were resuspended in PBS-heparin, and aliquots were spun in a cytocentrifuge (Shandon) for subsequent differential counting by Wright's staining (Leukostat II, Fisher Scientific).

Assessment of adherent cells. After removal of the fluids and chambers following the 6-hour exposure to antigen or buffer, sterile cover glasses were appended to the blister base at one antigen site and one buffer site for 15 minutes. The cover glasses were then removed and replaced with a second cover glass for 15 minutes. The first cover glass was stained with Wright's stain (Dif-Quik) for a differential leukocyte count. The second cover glass was assessed for the percent of activated eosinophils by using a monoclonal antibody that detects an eosinophil activation marker (EG₂, Pharmacia) and alkaline phosphatase-anti-alkaline phosphatase immunocytochemistry.

Biopsy of chamber site. In nine of 15 subjects, 3 mm punch biopsy specimens were obtained from one antigen challenge site and one buffer challenge site not previously exposed to coverslips. Frozen sections (4 to 6 µm) were fixed in acetone and underwent a standard alkaline phosphatase-anti-alkaline phosphatase immunohistochemical staining technique that used: (1) EG₁ monoclonal antibody (Pharmacia) to ECP as a marker of eosinophils; (2) EG₂ (monoclonal antibody against cleaved ECP, Pharmacia) as a marker of activated eosinophils; and (3) anti-lactoferrin antibody (Dako) for detection of this neutrophil granule protein, as previously described.^{3,5} Lactoferrin-positive cells were considered neutrophils. Anti-CD₃ monoclonal antibody (Leu4, Becton-Dickinson) was used as a marker of T lymphocytes. Coded slides were examined by two independent observers for number of positive cells per high power field (HPF); such cells were counted in 15 HPFs, starting in the most superficial part of the dermis in these biopsy specimens. In previous studies we had determined that the base of the blister induced by us was at the dermal-epidermal junction. After unroofing the blisters, the top of the dermis was at this dermal-epidermal junction. Semi-quantitative assessment of the extracellular deposition of lactoferrin and ECP was rated on a scale of 0 to 4⁺: 0, no staining; 1⁺, scattered areas of weak staining in the dermis; 2⁺, diffuse weak staining; 3⁺, diffuse moderate staining; 4⁺, intense diffuse staining.

Statistical analyses

Results were analyzed by paired *t* test after a two-way repeated-measures analysis of variance was performed for each of the parameters. Nonparametric data were analyzed by Wilcoxon rank-sum analysis.

RESULTS

Plasma cetirizine levels

Plasma cetirizine levels (available for 14 of 15 subjects), obtained just before dosing on the sixth day of drug or placebo administration, were 139 ± 25 ng/ml (range, 39 to 357 ng/ml) during drug administration and 0 during placebo administration.

Skin test responses

During administration of cetirizine, there was a significant inhibition of the wheal responses 20 minutes after intradermal injection of 100 PNU/ml and 10 PNU/ml antigen ($34\% \pm 4\%$ and $31\% \pm 4\%$, respectively). There was also a significant inhibition of the 20-minute flare responses to 100, 10, and 1 PNU doses of antigen ($46\% \pm 3\%$, $48\% \pm 3\%$, and $60\% \pm 4\%$, respectively). There was similar inhibition of the wheal and flare responses to histamine ($38\% \pm 4\%$ and $68\% \pm 3\%$, respectively) and codeine ($41\% \pm 3\%$ and $65\% \pm 4\%$, respectively) (Fig. 1). Despite the significant inhibition of the early skin test responses, there was no significant inhibition of either the late-phase induration or erythematous responses to either the 100 or 10 PNU/ml antigen doses. Indeed, the mean late-phase erythematous responses were slightly (but not significantly) greater during the cetirizine treatment period (Fig. 2).

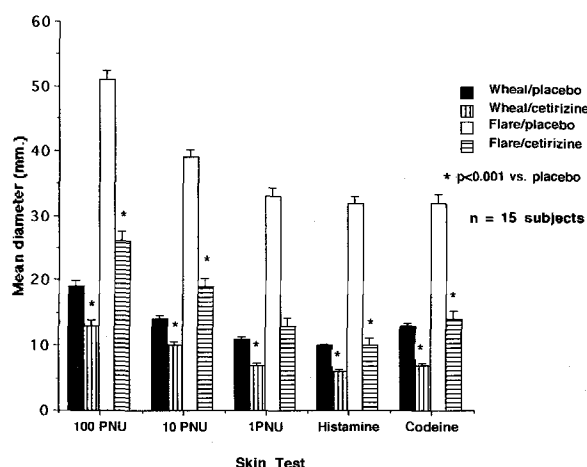


FIG. 1. Mean $\pm$ SEM diameter (in millimeters) of wheal and flare responses after intradermal injection of varying concentrations of pollen extract, histamine, and codeine during placebo and cetirizine treatment periods.

Skin chamber fluid mediators

As shown in Table I, the mean skin chamber histamine levels after the first hour of antigen challenge were somewhat higher during the cetirizine treatment period than during the placebo treatment period, but not significantly so (118 ± 16 vs 84 ± 17 , respectively; $p = \text{NS}$). This was due to very high histamine levels in two of the 15 subjects during the cetirizine treatment period. Both levels were significantly greater than those at the corresponding first-hour buffer challenge sites ($p < 0.001$). At sites challenged with antigen for the next 5 hours, during cetirizine and placebo administration periods, there were similar levels of histamine (44 ± 2 and 36 ± 8), of ECP (137 ± 18 and 97 ± 15), and of lactoferrin (19 ± 3 and 14 ± 5 , respectively). Each was significantly increased compared with levels of the same components at sites challenged with buffer in the same subjects ($p < 0.001$).

Cells in skin chamber fluids

As shown in Table II, there was significantly greater total cell accumulation in skin chambers at antigen-challenged sites compared with buffer-challenged sites during both cetirizine and placebo treatment periods; the total cell accumulation was actually significantly higher during cetirizine administration (23.2 ± 3.0 vs $16.7 \pm 3.4 \times 10^5$ cells). Most of the cells were neutrophils (83% to 90%) with significantly more at antigen sites than at buffer sites during both placebo and cetirizine treatment periods (mean $\pm$ SEM = 14.7 ± 0.30 vs $4.4 \pm 0.33 \times 10^5$ and 20.2 ± 0.37 vs 5.0 ± 0.09 , respectively; $p = 0.01$ for both). There was no significant difference in neutrophil accumulation patterns during placebo and cetirizine treatment periods. Likewise, the eosinophil accumulation at antigen sites was significantly greater than at buffer sites ($p = 0.01$) and not significantly different during both placebo and cetirizine treat-

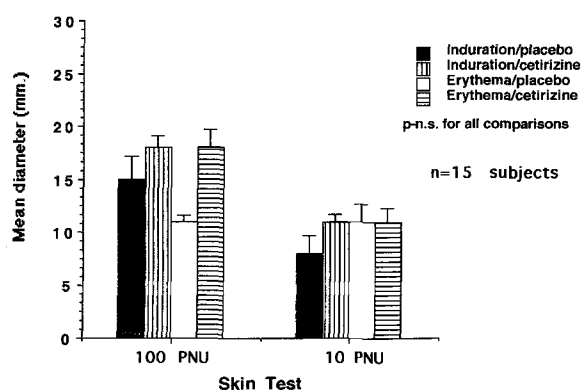


FIG. 2. Mean $\pm$ SEM diameter (in millimeters) of induration and erythema responses 6 hours after intradermal injection of pollen extract (100 PNU/ml and 10 PNU/ml) during placebo and cetirizine treatment periods.

ment (1.5 ± 0.31 vs 0.1 ± 0.04 and 1.7 ± 0.41 vs 0.1 ± 0.02 , respectively). The total mononuclear cell accumulation was also greater at antigen sites than at buffer sites (0.6 ± 0.11 vs 0.5 ± 0.11 ; 1.2 ± 0.28 vs 0.5 ± 0.09), though the percentage of these cells was greater at buffer sites during both placebo ($9\% \pm 2\%$ vs $2\% \pm 1\%$) and cetirizine ($8\% \pm 2\%$ vs $5\% \pm 1\%$) treatment periods ($p = 0.01$). Again, there was no difference in these mononuclear cell patterns between placebo and cetirizine treatment periods.

Adherent cells

To determine whether cetirizine might be affecting the inflammatory cells with adherent properties accumulating in the blister bases after 6 hours of antigen and buffer challenge, we evaluated the percentage of different cell types adhering to cover glasses appended to one antigen site and one buffer site for 15 minutes at the end of the 6-hour period. Results are shown in Table III. During both the cetirizine and placebo treatment periods, there was greater response at antigen sites than at buffer sites in: (1) the percent of eosinophils (13 ± 3 vs 5 ± 0.2 and 12 ± 2 vs 3 ± 1 , respectively, $p = 0.01$); (2) percent of activated (EG_2^+) eosinophils (1.4 ± 0.8 vs 0.3 ± 0.1 , $p \leq 0.05$ and 1.4 ± 0.6 vs 0.3 ± 0.1 , $p = 0.01$, respectively). There was also a lower percentage of mononuclear cells at antigen sites than at buffer sites (3 ± 1 vs 15 ± 4 , $p = 0.01$) only during placebo administration. Thus cetirizine did not reduce the percentage of adherent eosinophils or activated eosinophils accumulating at sites of antigen challenge.

Biopsy specimens of skin chamber sites

Nine of our 15 subjects consented to a biopsy of two skin chamber base sites (one antigen, one buffer) after the completion of the 6-hour antigen and buffer challenges. There was no significant difference in the numbers of neutrophils, eosinophils, mononuclear cells, or activated eosinophils at antigen sites compared with buffer sites; nor was there any difference in the findings

TABLE I. Skin chamber mediator levels in 15 subjects

	Placebo treatment period	Cetirizine treatment period
First hour histamine (ng/ml)		
Antigen site	84 ± 17*	118 ± 16*
Buffer site	1 ± 0.1	3 ± 2.2
Second to sixth hour histamine (ng/ml)		
Antigen site	36 ± 7.8*	44 ± 2.4*
Buffer site	1 ± 0.1	1 ± 0.4
ECP (ng/ml)		
Antigen site	97 ± 14.5*	137 ± 18*
Buffer site	15 ± 3.7	32 ± 7.4
Lactoferrin (μg/ml)		
Antigen site	14 ± 2.1*	18 ± 3.0*
Buffer site	5 ± 1.1	7 ± 3.0

Values are expressed as means ± SEM.

* $p < 0.001$ (antigen vs corresponding buffer sites).

during cetirizine versus placebo treatments (Table IV). However, we did observe a significant difference between antigen and buffer sites in extracellular deposition in the dermis of ECP during both the placebo and cetirizine periods (3.6 ± 0.2 vs 1.4 ± 0.2 and 3.3 ± 0.4 vs 1.3 ± 0.3 , respectively; $p = 0.01$ for both). Extracellular lactoferrin was also greater at antigen sites than at buffer sites during both placebo and cetirizine treatments (3.0 ± 0.3 vs 1.4 ± 0.2 and 3.7 ± 0.2 vs 1.0 ± 0.4 , respectively; $p = 0.01$ for both). Therefore there was no evidence that cetirizine affected: (1) the accumulation of cells in the dermis or (2) the activation of neutrophils and eosinophils, as evidenced by release of their granule products, into the extracellular dermis at sites of antigen exposure.

DISCUSSION

Our current findings in skin chamber studies during placebo administration were similar to those we previously reported, that is, significantly greater levels at antigen-challenged sites than at buffer-challenged sites in: (1) histamine levels (with a prolonged release pattern), (2) inflammatory cell accumulation (predominantly neutrophils), and (3) lactoferrin (released from neutrophil granules).³ New findings were a significantly greater accumulation of eosinophils and increased levels of ECP, most likely released from eosinophil granules.¹⁵ It is possible that some of the released ECP could have come from locally accumulated neutrophils, which could contain small amounts of ECP.²¹ The frequency but not the absolute number of mononuclear cells was significantly lower in the chamber fluids at antigen-challenged sites than at buffer-challenged sites, likely reflecting the relatively greater granulocyte exudation into skin chambers overlying the antigen sites. The lack of correlation between ECP levels and eosinophil number in individual fluids observed in this study likely reflects the fact that much of the ECP in such fluids reflected diffusion of

TABLE II. Cells in skin chamber fluids in 15 subjects

	Placebo treatment period	Cetirizine treatment period
Total cells		
Antigen site	16.7 ± 3.4*†	23.2 ± 3.0†
Buffer site	5.3 ± 1.5	5.6 ± 1.7
Neutrophils		
Antigen site	14.7 ± 0.3† (88)‡	20.2 ± 0.37† (87)
Buffer site	4.4 ± 0.3 (83)	5.0 ± 0.09 (90)
Eosinophils		
Antigen site	1.5 ± 0.31† (9)	1.7 ± 0.41† (7)
Buffer site	0.1 ± 0.04 (2)	0.1 ± 0.02 (2)
Mononuclear cells		
Antigen site	0.6 ± 0.11 (2)	1.2 ± 0.28 (5)
Buffer site	0.5 ± 0.11 (9)	0.5 ± 0.09 (8)

*Mean ± SEM cells × 10⁵.

† $p = 0.01$ (antigen vs corresponding buffer sites).

‡Percent of total cells is given in parentheses.

ECP from the allergic reactions in the underlying dermis. It is also possible that some of the eosinophils in the chamber had degranulated (releasing much ECP) to the point at which they were not detected by our usual Wright's staining.

Another new finding in this study during the placebo administration phase was a significantly greater percentage of both total and activated (EG₂⁺) eosinophils on cover glasses appended to the blister bases at antigen-challenged sites than at buffer-challenged sites. There was a lower percentage of mononuclear cells at antigen-challenged sites, whereas the relative frequency of neutrophils was not significantly different at antigen and buffer sites.

Biopsy specimens of the underlying dermis in antigen- and buffer-challenged sites in nine of the 15 subjects revealed no significantly greater number of neutrophils, eosinophils, activated eosinophils, or T lymphocytes at antigen-challenged sites. This lack of significant differences may reflect the increased prior emigration of these cells from the dermis at antigen-challenged sites onto the blister base surface and passage into the overlying skin chamber fluids. However, at antigen sites there was a significantly greater deposition of both extracellular lactoferrin and ECP than at buffer sites. These findings can be compared with previous reports by other groups.

There has been only one other study of granulocyte deposition in LPRs in which both biopsy and skin chamber techniques were used in subjects with cutaneous allergic reactions. Charlesworth et al.¹⁶ biopsied skin chamber bases at 8 hours after antigen challenge in five individuals. They found a significant increase in the frequency of basophils and eosinophils infiltrating the dermis and also appearing in the skin chamber fluids 8 to 12 hours after an initial antigen challenge. In a preliminary study of four subjects, Leifernan et al.¹⁷ found extracellular dermal deposition of an eosinophil granule protein (major basic protein) and neutrophil elastase in

TABLE III. Relative frequency of adherent cells in skin chamber bases

	Placebo treatment period	Cetirizine treatment period
Neutrophils (%)		
Antigen site	85 ± 2.5	82 ± 2.6
Buffer site	81 ± 3.7	85 ± 2.4
Eosinophils (%)		
Antigen site	11.6 ± 2.1*	13.0 ± 2.6*
Buffer site	3.3 ± 0.7	5.1 ± 0.2
EG2 ⁺ cells (%)		
Antigen site	1.4 ± 0.6†	1.4 ± 0.8†
Buffer site	0.3 ± 0.1	0.3 ± 0.1
Mononuclear cells (%)		
Antigen site	2.9 ± 1.1*	4.8 ± 1.7
Buffer site	14.8 ± 3.9	8.8 ± 2.1

**p* = 0.01 (antigen vs corresponding buffer sites).†*p* = <0.05 (antigen vs corresponding buffer sites).

biopsy specimens of LPR sites at 8 hours after intradermal antigen challenge. More recently, Tsicopoulos et al.¹⁸ found a significantly greater frequency of EG2⁺ and elastase-positive cells (neutrophils) in LPR sites than in control-challenged sites 6 hours after intradermal injection.

We found no inhibition of any of the humoral or cellular inflammatory events at antigen-challenged sites during cetirizine treatment. Indeed, the mean histamine, ECP, and lactoferrin levels, as well as the late-phase macroscopic responses were greater during cetirizine treatment than during placebo treatment, although not significantly so. This raises the question of whether cetirizine treatment might actually enhance some manifestations of allergic inflammation. However, we cannot draw such conclusions because of the variable effects of cetirizine on individual subjects. For example, during cetirizine treatment, histamine release was lower in six subjects and higher in nine subjects.

Our findings that cetirizine treatment did not inhibit mediator release or inflammatory cell responses can be compared with observations of others. Ting et al.¹⁹ found that 3 days of pretreatment with hydroxyzine (the parent compound of cetirizine) inhibited pollen antigen-induced histamine release during the first 30 minutes of challenge. They also found inhibition of antigen-stimulated mast cell granule exocytosis in biopsy specimens of sites 30 minutes after intradermal antigen during placebo treatment but not during hydroxyzine treatment. Michel et al.¹² found that cetirizine, 10 mg/day, for 4 days inhibited early spontaneous release of histamine and platelet activating factor into overlying skin chambers but had no effect on inflammatory cell accumulation in skin windows 24 hours after antigen challenge. One possible explanation for the previous findings described here and our observations in this study is that cetirizine may inhibit the initial interaction between the challenge antigen and the local mast cell, resulting in a transient inhibition of very early histamine release. However, there is no impairment of the release of histamine when

TABLE IV. Inflammatory responses in biopsy specimens of skin chamber bases

	Placebo treatment period	Cetirizine treatment period
Neutrophils/HPF		
Antigen site	11 ± 2.1	7.9 ± 2.6
Buffer site	9 ± 1.8	7.0 ± 1.3
Eosinophils/HPF		
Antigen site	1.1 ± 0.2	0.7 ± 0.1
Buffer site	1.3 ± 0.2	1.4 ± 0.3
Lymphocytes/HPF		
Antigen site	4.4 ± 1.0	3.3 ± 0.5
Buffer site	3.8 ± 0.9	3.4 ± 0.7
EC2 ⁺ cells/HPF (%)		
Antigen site	1.4 ± 1.1	1.1 ± 0.2
Buffer site	1.0 ± 0.3	1.1 ± 0.3
Extracellular deposition of ECP @		
Antigen site	3.6 ± 0.2*	3.3 ± 0.4*
Buffer site	1.4 ± 0.2	1.3 ± 0.3
Extracellular deposition of lactoferrin @		
Antigen site	3.0 ± 0.3*	3.7 ± 0.2*
Buffer site	1.4 ± 0.2	1.0 ± 0.4

Values are expressed as means ± SEM. Extracellular deposition is graded on a scale of 0 to 4⁺ (see text).

**p* = 0.01 (antigen vs buffer sites).

assessed 1 and 6 hours after initiation of antigen challenge, as in this study.

Charlesworth et al.¹⁰ found somewhat varying results in a series of studies of the effects of cetirizine on cutaneous allergic reactions. In their first study they reported that pretreatment with cetirizine, 20 mg/day, inhibited the release of prostaglandin D₂, but not histamine, into skin chambers overlying ongoing cutaneous allergic reactions.¹⁰ Accumulation of neutrophils and eosinophils in these chamber fluids was also reduced significantly, starting 8 hours after initiation of the antigen challenge. In a later study the same investigative group found that cetirizine treatment did not affect release of histamine or prostaglandin D₂ into skin chambers overlying ongoing cutaneous allergic reactions; however, eosinophil and neutrophil accumulation in such chambers was reduced 8 to 12 hours after initiation of the antigen challenge.¹¹ In both of these studies, cetirizine inhibited the immediate wheal and flare response but not the gross LPR to intradermal antigen.

Two other reports have described cetirizine inhibition of the accumulation of eosinophils in cutaneous allergic reactions. Fadel et al.⁶ compared the effects of a single 10 mg dose of cetirizine and placebo on the cellular response at intradermal antigen challenge sites in eight sensitive subjects; effects were assessed in skin windows applied to abraded sites for 24 hours. They found a partial (40%) inhibition of eosinophil influx without any effects on neutrophil or monocyte accumulation. In a similar type of study, these investigators later found a greater inhibition of eosinophil accumulation after 24

hours at sites of challenge with antigen (decreased 63%) or platelet activating factor (decreased 58%) in subjects pretreated with cetirizine, 20 mg/day, for 4 days.¹³ On the other hand, Varney et al.²⁰ found no effect of cetirizine treatment on neutrophil and eosinophil responses in biopsy specimens obtained 24 hours after intradermal antigen challenge.

The reasons for the differences in findings of the studies by Charlesworth et al.,^{10,11} Fadel et al.,¹³ and Varney et al.²⁰ and this study are not clear. Although plasma cetirizine levels were not mentioned in the previous reports, the doses used by those investigators and the degree of inhibition of wheal and flare reactivity were similar to those observed by us. The different findings could reflect different timing of the assessments in the different protocols. Charlesworth et al.¹¹ did not find inhibition of cellular influx by cetirizine until more than 8 hours after antigen challenge. Fadel et al.¹³ assessed inflammatory cell accumulation only after 24 hours. In comparison, we have focused our studies on the first 6 hours of antigen challenge when late-phase reactions are in the "early" developing stage.

Another possible explanation for the lack of cetirizine effects reported here by us is that the inflammatory responses we observed during placebo treatment of our subjects were much greater than those reported by Charlesworth et al.¹¹ in their placebo-treated subjects. The 10-fold greater histamine release and three- to fivefold greater inflammatory cell accumulation seen by us may not be suppressed by therapeutic doses of cetirizine.

In summary, we have found that cetirizine, a potent antihistamine, did not affect the gross or histologic events during the early (developing) late-phase allergic skin reactions. The complex events occurring during the first 6 hours of antigen challenge likely play important roles in the subsequent expression of late-phase reactions over the succeeding 24 hours. Observations by previous investigators that cetirizine may partially inhibit events occurring 8 hours after antigen challenge raise the possibility that cetirizine may interfere with the later enhancement of the early developmental expression of late-phase reactivity. Further investigation is required to differentiate cetirizine effects during the two periods.

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