

Cetirizine reduces inflammatory cell recruitment and ICAM-1 (or CD54) expression on conjunctival epithelium in both early- and late-phase reactions after allergen-specific challenge

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Background: Allergen-specific conjunctival challenge (ASCC) is a safe and reproducible experimental model of allergic conjunctivitis and a useful tool in the evaluation of effectiveness and possible mechanisms of action of drugs commonly used in the treatment of allergic diseases.

Objective: The protective effect of cetirizine on inflammatory changes after ASCC was assessed in 12 patients with rhinoconjunctivitis caused by *Parietaria judaica* in a double-blind study.

Methods: After a screening ASCC was performed, patients were randomized into two treatment groups; each patient was given cetirizine (oral tablets) 10 mg twice daily, or matching placebo for 3½ days in off-pollen season. Clinical evaluation (itching, hyperemia, lacrimation, and swelling of eyelids) and cytologic assessment (number of inflammatory cells in conjunctival scraping and evaluation of intercellular adhesion molecule-1 (ICAM-1)/CD54 expression on epithelial cells) were performed at baseline, 30 minutes (i.e., early-phase reaction [EPR]), 6 hours, and 24 hours (i.e., late-phase reaction [LPR]) after ASCC, before and after treatment.

Results: The EPR clinical events and the EPR total number of inflammatory cells were significantly reduced by cetirizine compared with placebo. The LPR clinical events and inflammatory cell recruitment were reduced by cetirizine in a similar manner. Both eosinophil and neutrophil numbers were decreased by active drug in EPR and LPR. Furthermore, ICAM-1/CD54 expression was significantly reduced by cetirizine in both the EPR and LPR compared with placebo.

Conclusions: This study shows that cetirizine has a protective effect on clinical and cellular EPR and LPR events (including ICAM-1/CD54 expression on epithelium) induced by ASCC. (J ALLERGY CLIN IMMUNOL 1995;95:612-21.)

Key words: Allergic inflammation, adhesion molecules, ICAM-1/CD54, antiallergic drugs, cetirizine, allergic conjunctivitis

The allergen-specific conjunctival challenge (ASCC) is a safe and reproducible experimental model of allergic conjunctivitis.^{1,2} When high allergen dosages are used, the allergic response is

characterized by a two-phase reaction, namely the early-phase reaction (EPR) and the late-phase reaction (LPR).³ In contrast to nasal,^{4,5} bronchial,^{5,6} and skin provocation models,⁵ no quiescent

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Abbreviations used

APAAP:	Alkaline phosphatase–monoclonal anti-alkaline phosphatase
ASCC:	Allergen-specific conjunctival challenge
AUR:	Allergen unit by RAST
EPR:	Early-phase reaction
ICAM-1:	Intercellular adhesion molecule-1
LPR:	Late-phase reaction
mAb:	Monoclonal antibody

(symptom-free) interval exists in the conjunctival model, probably because of the high allergen dose used in ASCC.³ ASCC evaluation criteria included clinical examination and cytologic assessment in the lacrimal fluid.^{3,7-9} Furthermore, a sensitive immunocytochemical technique has recently allowed us to demonstrate intercellular adhesion molecule-1 (ICAM-1)/CD54 expression on conjunctival and nasal epithelium after allergen-specific challenge in allergic subjects.^{10,11} ICAM-1/CD54, a member of the immunoglobulin supergene family, is the main physiologic ligand of the leukocyte integrin lymphocyte function related antigen-1, which is widely expressed on leukocytes (including eosinophils).^{12,13} The lymphocyte function related antigen-1/ICAM-1 intercellular adhesion system is involved in immunologic reactions requiring intercellular contact, such as in allergic inflammation.¹⁴

Effectiveness and possible mechanisms of action of drugs used in the treatment of allergic disease may be evaluated throughout the modulation of the events consequent to ASCC, and in this regard, we have already demonstrated a preventive effect of different pharmacologic compounds, such as antihistamines (e.g., terfenadine and loratadine) and steroids (e.g., deflazacort) in ASCC.^{7,8,15}

Cetirizine, a carboxylated metabolite of hydroxyzine, is a potent histamine H₁-receptor antagonist, devoid of the central nervous system depressant effects of other antihistamines¹⁶ and effective in treating allergic rhinoconjunctivitis¹⁷ and chronic idiopathic urticaria.¹⁸ Cetirizine exerts antiallergic activity that prevents clinical changes consequent to allergen-specific challenge performed at conjunctival,^{19,20} nasal,²⁰ bronchial,²¹⁻²³ and cutaneous^{20,24} levels. Although cetirizine does not inhibit in vivo immediate antigen-induced histamine release in atopic subjects, it is capable of inhibiting the late release, 24 hours after prolonged contact of the skin with the antigen.²⁵ Cetirizine reduces

eosinophil²⁵⁻²⁷ and neutrophil²⁷ infiltration to the site of allergic reaction and platelet activating factor (PAF) release.²⁸ In vitro, cetirizine reduces eosinophil chemotaxis to PAF^{29,30} and is devoid of effect on lymphocyte proliferation.³¹

This study was designed to investigate the possible effects of cetirizine on clinical and cytologic changes (including ICAM-1/CD54 expression on epithelial cells) both during EPR and LPR, consequent to conjunctival challenge in allergic subjects.

METHODS

Study design

The study was a placebo-controlled, double-blind, randomized, parallel-group trial. *Parietaria judaica*-sensitive patients with allergic rhinoconjunctivitis were studied in the off-pollen season during remission of symptoms.

Conjunctival challenge, clinical evaluation, and cytologic assessment (including ICAM-1/CD54 expression on conjunctival epithelium) were performed as described in our previous reports.^{7,8,10,15} A preliminary ASCC ("screening test") was performed weekly for 3 weeks to identify patients with LPR to *P. judaica* extract at different dilutions.

P. judaica extract (kindly provided by Bayropharm-DHS, Milan, Italy), the same as that used for skin prick testing, stored as a lyophilized powder at 4°C, was reconstituted in the diluent, saline human albumin (0.03%) solution (kindly provided by Bayropharm-DHS) within 24 hours before the test. The concentration was expressed as Allergen Units by RAST (AUR) per milliliter (AUR is a standardized Bayropharm-DHS unit). Eight different dilutions were used during screening ASCC: 20, 60, 200, 600, 1200, 2000, 3000, and 5000 AUR/ml. The same batch of allergen was used throughout the study. Ten microliters of diluted allergen was instilled into the lower conjunctival sac in the right eye. Saline albumin diluent, used to dilute the allergen, was instilled into the contralateral eye to exclude possible nonspecific reactivity. The initial allergen concentration was the same as that used in the skin end point test. During the following weeks, the next more concentrated allergen dilution was used until a late clinical reaction was elicited (total symptom score >2 was considered positive for LPR, as previously described), and therefore the LPR provoking dose was defined.^{7,8}

After preliminary screening, selected patients (12 of 15) were randomly assigned to two parallel treatment groups; each patient received a daily oral dose of 10 mg of cetirizine or placebo, twice daily, respectively, in the morning and in the evening for 3½ days. Patients underwent ASCC with allergen provoking dose before and after treatment. The last medication dose was administered in the morning, 3 hours before ASCC.

Clinical conjunctival evaluation and cytologic assessment were performed at baseline, 30 minutes, and 6 and

24 hours after challenge. The clinical reaction to the allergen was assessed by evaluating conjunctival hyperemia, lacrimation, itching or burning, and swelling of eyelids; a score was assigned according to an arbitrary 4-point rating scale, from 0 to 3 (0 = absent, 1 = mild, 2 = moderate, 3 = severe). A total symptom score was calculated as the sum of individual symptom scores.^{9, 10, 16, 24}

Concomitantly, conjunctival scrapings for cytologic and immunocytochemical assessment were obtained from both eyes. After topical anesthesia (ossibuprocaine 4 mg/ml, 1 drop each eye) was induced, the upper tarsal conjunctiva was scraped with a sterile Kimura spatula. Specimens were spread on glass slides, air-dried, stained with May-Grunwald Giemsa dye, and examined by microscope (Leitz Laborlux D microscope [Wild Microscopes, Rockleigh, N.J.] $\times 250, \times 500$). The slides were separately examined by two investigators blinded to the identity of the samples. The number of inflammatory cells (i.e., neutrophils, eosinophils, lymphocytes, and monocytes) was counted individually and as a total number of cells in each microscopic field; data are expressed as a mean of 10 fields.^{7, 8, 10, 15} At least 200 cells for each specimen were examined.

The immunoenzymatic alkaline phosphatase–monoclonal anti-alkaline phosphatase (APAAP) complex procedure was used to detect ICAM-1/CD54 expression on epithelium. Specimens were air-dried at room temperature for 30 minutes and submitted to appropriate dilution of purified ICAM-1/CD54 monoclonal antibody (mAb) (1 mg/ml, 84H10, IgG₁; Immunotech, Marseille, France) or anti-cytokeratin mAb (DAKO, Milan, Italy). After washing in phosphate-buffered saline (pH = 7.6), samples were incubated with rabbit anti-mouse-immunoglobulin, followed by APAAP complex. Afterwards, specimens were incubated in substrate solution containing basic new fuchsin, naphthol as biphosphate, and levamisole as an inhibitor of endogenous alkaline phosphatase (Sigma Chemical Company, St. Louis, Mo.). In control samples either mAb or anti-mouse immunoglobulin was omitted. All preparations were counterstained with Carazzi's hematoxylin and examined by two investigators blinded to the identity of the samples. ICAM-1/CD54 expression on epithelial cells was expressed according to a 5-point rating scale, from 0 to 4 where 0 = no positive cells, 1 = mild positivity on 25% of epithelial cells, 2 = mild positivity on 75% of epithelial cells, 3 = intense positivity on 75% of epithelial cells, and 4 = very intense positivity on all epithelial cells, as previously described.¹⁰

All possible adverse events were spontaneously reported by the patients and carefully evaluated by physicians.

Study subjects

Fifteen subjects with *P. judaica* sensitivity and seasonal allergic rhinoconjunctivitis (10 men and 5 women, aged 18 to 43 years) were screened in the off-pollen

season. All patients had a history of pollen allergy for at least two previous seasons and positive skin prick test and RAST responses to the specific pollen. No patient had skin test sensitivity to any other allergen, and no patient had symptoms at any other time. All patients were free of other ocular diseases, and none of them wore contact lenses. Patients with previous documented allergy to the studied drug, women of childbearing potential, and women who were lactating were excluded from the study.

After screening, 12 of 15 patients, 7 men and 5 women, aged 18 to 39 years (mean age, 27.2 years) were included in the trial and randomized to double-blind treatment.

No topical or systemic drugs were allowed for 1 month before the study or during the study period. All subjects gave informed consent, and the trial was approved by the department ethical committee.

Statistical analysis

Statistical analysis of results was performed by Wilcoxon signed rank test for paired data to compare differences within groups and by Mann-Whitney U test for independent data to compare differences between groups; *p* values of less than 0.05 were considered statistically significant.

RESULTS

Pretreatment ASCC

Baseline. Table I reports clinical features of subjects included in the study. All patients were free of symptoms; few inflammatory cells (mainly neutrophils, 1 to 2 per microscopic field) were detected in 5 of 12 enrolled subjects; no ICAM-1/CD54 expression was evident in any patient. A relevant number of epithelial cells was obtained by conjunctival scrapings (as many as 15 to 30 cells per microscopic field at $\times 250$); they all exhibited positive staining with anti-cytokeratin mAb. Therefore at baseline, the cetirizine group and the placebo group did not show any difference as far as either clinical and cellular parameters were concerned (Figs. 1 to 4). Moreover, the topical anesthesia with ossibuprocaine did not affect any clinical or cellular parameter in any of the studied subjects, as previously described.^{7, 8, 10, 15}

EPR (30 minutes). All subjects showed an immediate hypersensitivity conjunctival reaction within 5 minutes after allergen challenge, at concentrations ranging from 2000 to 5000 AUR/ml (Table I). The EPR was characterized by conjunctival hyperemia, lacrimation, burning-itching, and swelling of eyelids; the reaction was significant compared with baseline and contralateral eye (*p* < 0.0005) (Fig. 1). A significant number of inflammatory cells (i.e., 15 to 25 neutrophils per micro-

TABLE I. Clinical features of allergic subjects

Patient No.	Age (yr)	Sex	SPT response to <i>P. judaica</i>	RAST response to <i>P. judaica</i> (class)	Skin test end point (AUR/ml)	PD (AUR/ml)
Cetirizine (10 mg/ b.i.d.) group						
2	18	F	+++	III	60	3000
4	25	M	++++	IV	10	3000
6	31	M	++++	IV	20	2000
8	19	M	+++	III	20	5000
9	43	F	++++	IV	60	2000
12	38	M	++++	IV	60	3000
Placebo group						
1	22	F	++++	IV	60	2000
3	27	M	+++	III	20	2000
5	33	M	++++	IV	60	5000
7	39	F	++++	IV	60	3000
10	19	F	+++	III	60	3000
11	35	M	+++	III	20	5000

SPT, Skin prick test; PD, provocation dose for LPR; b.i.d., twice daily.

scopic field, 3 to 9 eosinophils, 2 to 6 lymphocytes, and 0 to 5 monocytes) was detected in challenged eyes compared with control eyes and baseline conditions ($p < 0.001$) (Figs. 2 and 3). All subjects showed a remarkable ICAM-1/CD54 expression compared with baseline and contralateral eye ($p < 0.0005$) (Fig. 4).

LPR (6 hours). The intensity of the ocular reaction was decreased and was characterized mainly by hyperemia and swelling of eyelids, significantly evident compared with contralateral eye and baseline ($p < 0.001$) (Fig. 1). Together with neutrophils (7 to 17 cells per microscopic field), a significant infiltration of eosinophils (8 to 16 cells), lymphocytes (3 to 7 cells), and monocytes (1 to 6 cells) was detected compared with baseline ($p < 0.001$) (Figs. 2 and 3). ICAM-1/CD54 expression on epithelial cells was present; the intensity was significantly lower compared with the observation at 30 minutes ($p < 0.05$) but still highly significant compared with baseline ($p < 0.001$) (Fig. 4).

LPR (24 hours). Swelling of eyelids and mild hyperemia persisted, significantly evident compared with baseline ($p < 0.01$) (Fig. 1); neutrophils (2 to 5 cells per microscopic field), lymphocytes (3 to 7 cells), eosinophils (2 to 4 cells), and monocytes (0 to 3 cells) persisted in conjunctival scrapings ($p < 0.01$) (Figs. 2 and 3). ICAM-1/CD54 expression on epithelial cells was decreased but still present compared with baseline conditions and contralateral eye ($p < 0.01$) (Fig. 4).

In regard to all studied parameters, no difference was demonstrated between the two studied groups (cetirizine-treated and placebo-treated) 30 minutes, 6 hours, and 24 hours after challenge and before the trial.

Posttreatment ASCC

After treatment, all patients were rechallenged with the same provoking dose (ranging from 2000 to 5000 AUR/ml) as that used before treatment (Table I). Compared with prechallenge ASCC, similar numbers of epithelial cells (together with spared inflammatory cells) were obtained from conjunctival scrapings.

EPR (30 minutes). Clinical changes were significantly less severe in the cetirizine-treated group compared with pretreatment values ($p < 0.032$) and values in the placebo-treated group ($p < 0.004$) (Fig. 1). Cellular infiltrate (total number of inflammatory cells) was significantly reduced by cetirizine compared with pretreatment values ($p < 0.024$) and values in the placebo-treated group ($p < 0.013$) (Fig. 2).

According to analysis of single cell type, eosinophils were significantly reduced by cetirizine compared with pretreatment values ($p < 0.05$) and values in the placebo-treated group ($p < 0.032$), as were neutrophils ($p < 0.03$ and $p < 0.036$, respectively) (Fig. 3). No effect regarding lymphocytes and monocytes was observed (Fig. 3). ICAM-1/CD54 expression on the epithelium

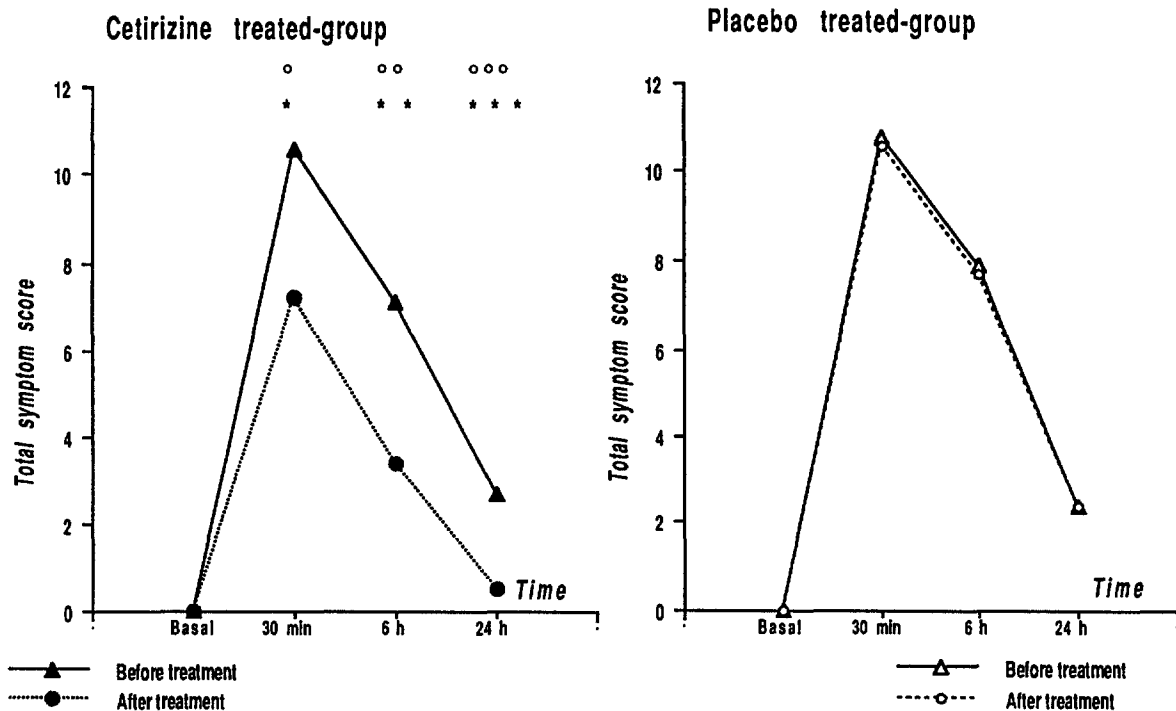


FIG. 1. Median values of total symptom scores before and after treatment and before and after ASCC. Total symptom scores were significantly increased in all patients 30 minutes ($p < 0.0005$), 6 hours ($p < 0.001$), and 24 hours ($p < 0.01$) after ASCC (Wilcoxon signed rank test). Wilcoxon test: $\circ p = 0.032$ before treatment versus after treatment (95% confidential limits of median differences = 0-7); $\infty p = 0.021$ before treatment versus after treatment (95% confidential limits of median differences = 0-8); $\infty\infty p = 0.017$ before treatment versus after treatment (95% confidential limits of median differences = -1-5). Mann-Whitney test: $*p < 0.004$ cetirizine versus placebo; $**p < 0.0002$ cetirizine versus placebo; $***p < 0.0003$ cetirizine versus placebo.

was significantly reduced by cetirizine compared with both pretreatment values ($p < 0.04$) and values in the placebo-treated group ($p < 0.008$) (Fig. 4).

LPR (6 hours). Clinical events were significantly less severe in the cetirizine-treated group compared with both pretreatment values ($p < 0.021$) and values in the placebo group ($p < 0.0002$) (Fig. 1). Total cell numbers were significantly reduced in the cetirizine group compared with both pretreatment values ($p < 0.017$) and values in the placebo group ($p < 0.002$) (Figs. 2 and 3). Eosinophils were reduced in the cetirizine group with respect to both pretreatment values ($p < 0.01$) and values in the placebo group ($p < 0.0001$) (Fig. 3). Neutrophils were also reduced in the cetirizine group compared with both pretreatment values ($p < 0.012$) and values in the placebo group ($p < 0.0015$) (Fig. 3). Lymphocytes were reduced in the cetirizine group with respect to both pretreatment values ($p < 0.026$) and values in the placebo group ($p < 0.008$) (Fig. 3). Monocytes were reduced in the

cetirizine group compared with both pretreatment values ($p < 0.05$) and values in the placebo group ($p < 0.032$) (Fig. 3). ICAM-1/CD54 expression was reduced in the cetirizine group compared with both pretreatment values ($p < 0.017$) and values in the placebo group ($p < 0.017$) (Fig. 4).

LPR (24 hours). Clinical scores were diminished in the cetirizine group compared with both pretreatment values ($p < 0.017$) and values in the placebo group ($p < 0.0003$) (Fig. 1). The total number of inflammatory cells was reduced in the cetirizine group compared with both pretreatment values ($p < 0.002$) and values in the placebo group ($p < 0.002$) (Fig. 2). Eosinophil counts were reduced in the cetirizine group compared with both pretreatment values ($p < 0.012$) and values in the placebo group ($p < 0.001$) (Fig. 3). Neutrophil infiltration was reduced in the cetirizine group compared with both pretreatment values ($p < 0.04$) and values in the placebo group ($p < 0.001$) (Fig. 3). Lymphocyte accumulation was diminished in the cetirizine group compared with both pre-

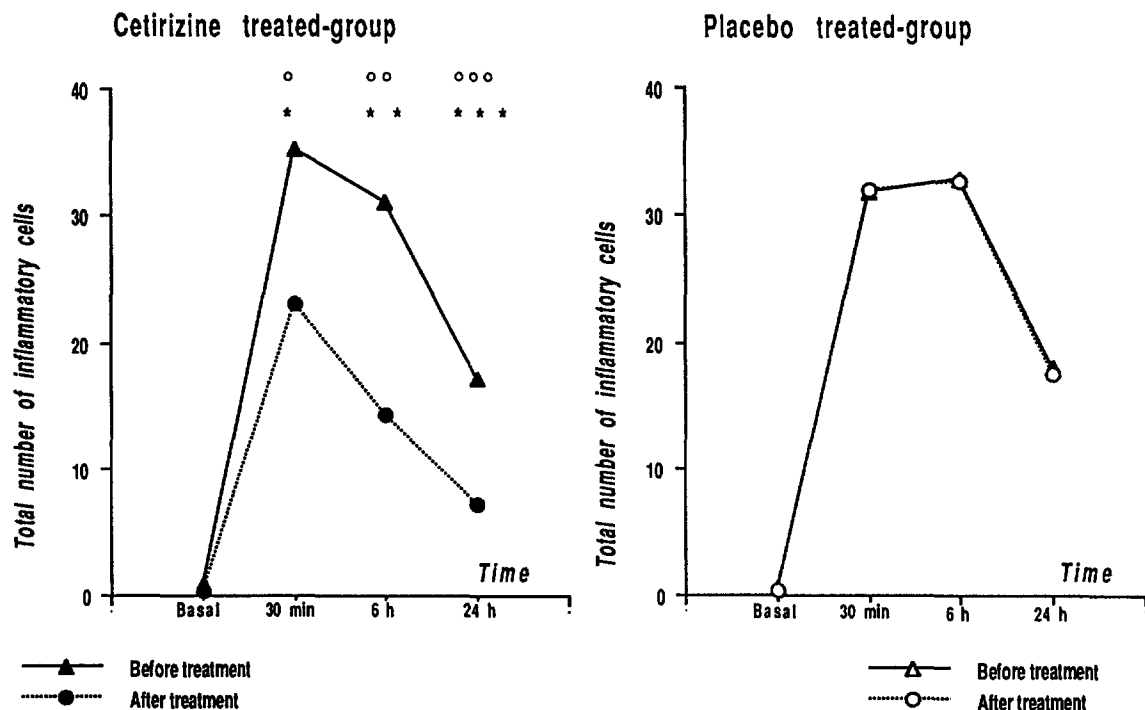


FIG. 2. Median values of total numbers of inflammatory cells before and after treatment and before and after ASCC. Total numbers of inflammatory cells were significantly increased in all patients 30 minutes ($p < 0.001$), 6 hours ($p < 0.001$), and 24 hours ($p < 0.01$) after ASCC (Wilcoxon signed rank test). Occasionally, a few subjects showed some inflammatory cells (1 to 2 per microscopic field). Wilcoxon test: $\circ p = 0.024$ before treatment versus after treatment (95% confidential limits of median differences = 1-22); $\infty p = 0.017$ before treatment versus after treatment (95% confidential limits of median differences = -1-35); $\infty\infty p = 0.002$ before treatment versus after treatment (95% confidential limits of median differences = 0-20). Mann-Whitney test: $*p < 0.013$ cetirizine versus placebo; $**p < 0.002$ cetirizine versus placebo; $***p < 0.002$ cetirizine versus placebo.

treatment values ($p < 0.032$) and values in the placebo group ($p < 0.02$) (Fig. 3). Monocyte counts were reduced in the cetirizine group compared with both pretreatment values ($p < 0.042$) and values in the placebo group ($p < 0.041$) (Fig. 3). Finally, ICAM-1/CD54 expression was reduced in the cetirizine group compared with both pretreatment values ($p < 0.04$) and values in the placebo group ($p < 0.032$) (Fig. 4).

Cellular LPR changes (including ICAM-1/CD54 expression on conjunctival epithelium) appeared more affected than EPR changes in the cetirizine-treated group ($p < 0.002$). The placebo-treated group did not demonstrate any statistically significant difference after treatment.

Side effects

Both cetirizine and placebo were well tolerated, and no adverse events were reported at any time during the study.

DISCUSSION

The efficacy and therapeutic value of antihistamines in the treatment of allergic diseases has been extensively demonstrated.³² Particularly, the new antihistaminic compounds are characterized by high receptor selectivity, potent activity, long half-life, and few (if any) sedative effects.³² In addition, in experimental allergen-specific challenges, the new antihistamines (such as terfenadine, astemizole, loratadine, and cetirizine) proved to be effective in dampening or blocking the appearance of the EPR phenomena at conjunctival,^{7,15,19} nasal,^{33,34} and cutaneous levels.^{33,34} Thus these compounds have been shown to have antiallergic activity.³⁵

On the other hand, few studies have been performed to investigate the antihistamine protection on LPR phenomena, because late-phase events are strictly connected to the inflammatory response consequent to allergic reaction, and it should be emphasized that LPR is directly related to the

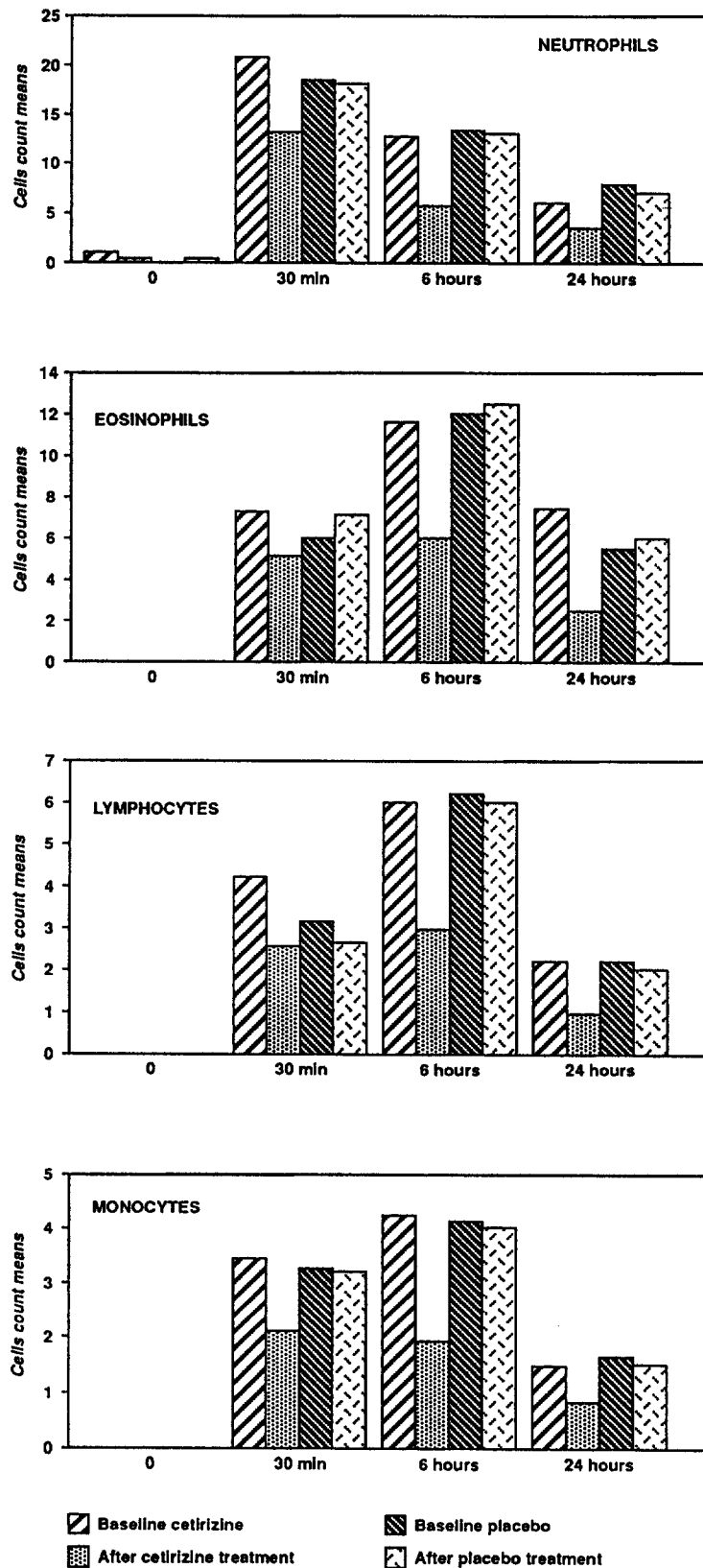


FIG. 3. Mean cell counts of neutrophils, eosinophils, lymphocytes, and monocytes in the cetirizine and placebo groups during ASCC before and after treatment.

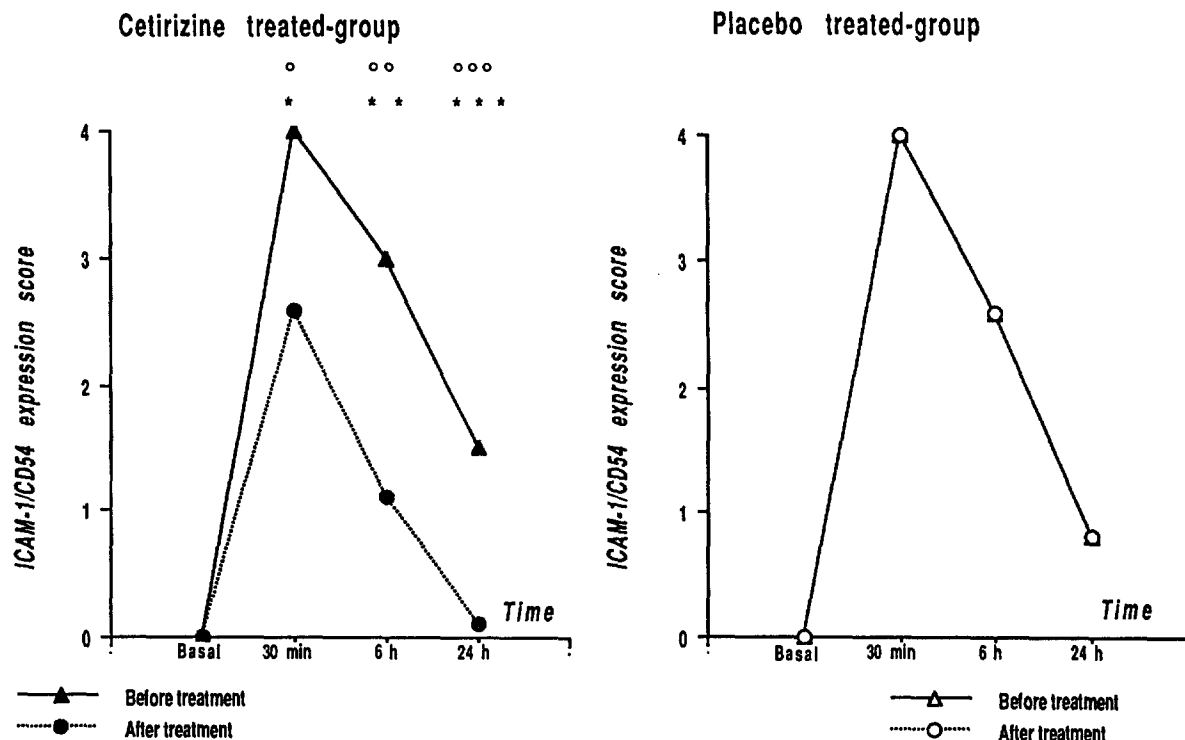


FIG. 4. Median values of total scores of ICAM-1/CD54 expression on epithelial cells before and after treatment and before and after ASCC. Deviation of individual values from the median was less than 1. ICAM-1/CD54 expression on conjunctival epithelium was significantly increased in all patients 30 minutes ($p < 0.0005$), 6 hours ($p < 0.001$), and 24 hours ($p < 0.01$) after ASCC (Wilcoxon signed rank test). Wilcoxon test: $^{\circ}p = 0.04$ before treatment versus after treatment (95% confidential limits of median differences = 0-2); $^{\infty}p = 0.017$ before treatment versus after treatment (95% confidential limits of median differences = 1-3); $^{\infty\infty}p = 0.0024$ before treatment versus after treatment (95% confidential limits of median differences = 0-2). Mann-Whitney test: $^*p < 0.008$ cetirizine versus placebo; $^{**}p < 0.0017$ cetirizine versus placebo; $^{***}p < 0.032$ cetirizine versus placebo.

clinical status of allergic disease and to the degree of hyperresponsiveness.^{5,6} Thus LPR phenomena should be regarded as more similar to a natural clinical allergic reaction than EPR phenomena.

As far as cetirizine is concerned, Schoeneich and Pecoud¹⁹ have already demonstrated that cetirizine reduces allergen-induced ocular itching and redness and increases the allergen threshold dose.¹⁹ Fadel et al.²⁵ showed that cetirizine, 10 mg in a single oral dose, blocks the wheal and flare reaction induced by intradermal injection of an antigen in atopic subjects and the accumulation of eosinophils observed 24 hours after challenge. They also showed that cetirizine inhibits the accumulation of eosinophils, when platelet activating factor is used as stimulus of the skin in allergic subjects.²⁵ Cetirizine does not prevent mediator release by activated mast cells.²⁴ Rédiér et al.²³ showed that cetirizine is able to inhibit eosinophil

recruitment caused by allergen challenge at the bronchial level.²³

In this study, we investigated the possible effects of cetirizine on EPR and LPR changes occurring after ASCC by following a schedule at a high dosage (10 mg twice daily). As described by several authors, including ourselves, ASCC results in the development of an inflammatory process. Although recruitment of inflammatory cells to the site of allergic reaction is well documented, activation of such cells and the interaction with epithelial cells appear to be crucial in the allergic reaction and involve a large number of morphologic and functional changes, including changes in adhesion machinery.^{10, 13, 36}

We have recently provided evidence of ICAM-1/CD54 expression on conjunctival and nasal epithelium after allergen-specific challenge.^{10, 11} ICAM-1/CD54 expression on epithelium occurred

as an early event (simultaneously with the inflammatory infiltrate), persisted during LPR, and vanished 24 hours after the challenge, as previously described.^{10,11} The occurrence of this phenomenon after allergen exposure appeared to be restricted to allergic subjects.^{10,11} ICAM-1/CD54 has been demonstrated to be effectively synthesized in epithelial cells by Kvale et al.³⁷ who found ICAM-1/CD54 mRNA in a colonic epithelial cell line on stimulation with interferon- γ as determined by Northern blot analysis. We have recently demonstrated ICAM-1/CD54 mRNA in both a conjunctival cell line (W-K) and conjunctival epithelial cells of allergic subjects on allergen stimulation by *in situ* hybridization technique (manuscript in preparation). In addition, specific ICAM-1/CD54 mRNA has been demonstrated in conjunctival epithelium in allergic subjects, even before allergen-specific challenge, without protein expression. Thus it is likely that still unknown factors (e.g., mediators other than histamine) may rapidly induce epithelial ICAM-1 expression, acting at the posttranscriptional level (Pesce et al. Manuscript in preparation).

Several findings indicate that the adhesion machinery plays a central role in allergic inflammation and suggest that maneuvers designed to decrease adhesion of inflammatory cells may be a useful therapeutic approach. This prompted us to evaluate not only clinical events and cellular infiltration but also ICAM-1/CD54 expression on conjunctival epithelium after challenge and to monitor the EPR and LPR before and after a short-term treatment with cetirizine.

The results showed that cetirizine exerts a protective effect on both EPR and LPR (up to 24 hours after challenge), resulting in a decrease of symptom severity and cellular infiltrate. Particularly, LPR events appear to be more affected by cetirizine treatment than EPR events. In addition, analysis of single cell count demonstrated that eosinophil infiltration was significantly inhibited both in EPR and LPR, though more in the latter, according to previous reports.^{23, 25, 26, 27} Neutrophil accumulation is reduced as well, in both EPR and LPR. Mononuclear cell (i.e., lymphocytes and monocytes) infiltration is inhibited by cetirizine during LPR only. Thus these data fit well with the known ability of cetirizine to prevent granulocyte infiltration to the site of allergic reaction.^{23, 25-28}

Notably, ICAM-1/CD54 expression on epithelial cells appears to be more reduced in the LPR than in the EPR. This finding is of great interest and may reflect a prolonged antiinflammatory effect of cetirizine,

which could in turn interfere with interactions between inflammatory cells (particularly eosinophils) and epithelium throughout surface molecules. The mechanism of action by which cetirizine inhibits ICAM-1/CD54 expression is uncertain because the effect of histamine or other mediators or cytokines involved in the allergic reaction on ICAM-1/CD54 expression is still not fully clarified. By performing conjunctival provocation tests with histamine, we have recently demonstrated that histamine is unable to induce either local inflammatory or ICAM-1/CD54 expression on conjunctival epithelium in pollen-sensitive patients (out of pollen season), as well as in healthy volunteers.³⁸ Consequently, it is likely that cetirizine might interfere with ICAM-1/CD54 expression through a mechanism other than histamine-mediated.

Finally, epithelial cells expressing ICAM-1/CD54 should no longer be considered merely by-standers in the development of an allergic reaction.³⁹ They are likely to play an active role in the inflammatory response. In fact, eosinophils express lymphocyte function related antigen-1 adhesion molecule, which is the natural ICAM-1/CD54 receptor, and it is well known that eosinophil infiltration is the most typical expression of an allergic inflammatory reaction.⁴⁰

In conclusion, it is possible to hypothesize that cetirizine modulates the *in vivo* cellular infiltration and consequent tissue damage in part through modulating effect on the adhesion machinery.

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