

Cetirizine inhibits skin reactions but not mediator release in immediate and developing late-phase allergic cutaneous reactions. A double-blind, placebo-controlled study

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Summary

Background Recent reports have indicated cetirizine, a potent H₁-receptor antagonist, to possess a number of anti-inflammatory effects, e.g. inhibition of mast cell degranulation and inhibition of leucocyte migration and activation.

Objective The aim of this study was to compare the effects of cetirizine on skin responses and mediator release in intact skin in immediate and developing late-phase allergic reactions by microdialysis technique.

Methods Cetirizine 10 mg once daily or matching placebo were administered to 10 atopic subjects for 6 days followed by a 2-week washout in a randomized, double-blind, placebo-controlled, cross-over trial. Immediate skin test responses to allergen, codeine, and histamine and late-phase reactions to allergen were assessed. The time course of extracellular levels of inflammatory mediators in intact skin were monitored by microdialysis techniques using 2 kDa and 3 MDa cut-off fibers, respectively.

Results Cetirizine significantly reduced immediate weal and flare reactions to allergen, codeine, and histamine. Injection of allergen, but not buffer controls, induced a significant release of histamine, tryptase, prostaglandin D₂, total protein, and eosinophilic cationic protein. No significant increase of leukotriene B₄ and myeloperoxidase was observed. Cetirizine inhibited early total protein extravasation by 40%, but this did not reach a significant level. None of the inflammatory mediators were significantly inhibited by cetirizine. Cetirizine significantly reduced the late-phase skin induration to allergen by approximately 30%.

Conclusion Cetirizine potently reduced skin responses in immediate allergic reactions without inhibition of early mediators. These data indicate cetirizine to be a potent H₁-receptor antagonist with no effect on mast cell activation. It did not inhibit any of the late-phase mediators, but it reduced the late skin reaction. These data suggest that mediators other than those actually measured may play a significant role in the clinical late-phase reaction.

Keywords: cetirizine, immediate hypersensitivity, microdialysis, mast cells, late-phase reaction, inflammatory mediators, skin tests.

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Introduction

Cetirizine is a potent and rapid acting, non-sedating H₁-receptor antagonist (antihistamine) with established clinical

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efficacy in allergic diseases [1]. During the last decade, several investigations have suggested that cetirizine and some other antihistamines possess a number of anti-inflammatory effects beyond that of H₁-receptor blockade. *In vitro* cetirizine inhibits the release of mast cell-derived mediators such as histamine and prostaglandin D₂ from mast cells [2], and reduces eosinophil chemotaxis and activation [3,4]. However, reports on its anti-inflammatory effects in human skin *in vivo* are conflicting. Histamine release by allergen and codeine phosphate is not inhibited by cetirizine [5–7]. However, cetirizine is the carboxylated metabolite of hydroxyzine, the latter being an inhibitor of histamine release in human skin [8,9]. An inhibitory effect of cetirizine on PGD₂ synthesis *in vivo* is controversial [6,10]. Cetirizine may inhibit also the late allergic skin response. Several studies have demonstrated reduced numbers of eosinophils and neutrophils in cutaneous late-phase reactions as assessed by skin chamber and window methods [5,6,11,12]. In contrast, the majority of skin biopsy studies has failed to show suppressive effects of cetirizine on eosinophil recruitment and activation as well as the cellular trafficking and mediator release from other inflammatory cells [13–16].

The conflicting results obtained so far may be due to the methods applied. None of the previously applied methods allow direct comparison of skin responses and mediator release in intact skin, and they do not allow assessment of the time course of changes of mediator levels in intact skin. We have previously used a microdialysis technique to analyse the release of low molecular weight mediators in human skin [17,18]. Recently, high molecular weight dialysis fibers became available [19,20]. The purpose of this study was to evaluate the effects of cetirizine in daily recommended dosages in steady-state conditions on immediate- and late-phase allergic cutaneous responses and mediator release using cutaneous microdialysis techniques.

Materials and methods

Subjects

Ten subjects (7 males, 3 females) participated in the study. Their mean age was 26 years (range 23–37). They were all allergic to timothy grass pollen (*Phleum pratense*), the allergy diagnosis being based upon relevant symptoms, positive skin prick tests, and specific IgE antibodies against timothy grass (RAST class > 2; CAP system; Pharmacia, Uppsala, Sweden). None of the subjects took any medication known to interfere with mast cell degranulation and/or interpretation of skin test responses. The study was performed several months after the end of the grass pollen season. Written and oral consent was obtained from the

subjects, and the study was approved by the local ethics committee and the Danish Board of Health.

Study design and drug treatment regimen

The study was performed as a randomized, double-blind, placebo-controlled, cross-over trial in one centre. The eligibility criteria were assessed and informed consent was obtained at prestudy visits. The investigative procedures were performed at two visits after 6 days of treatment with 10 mg once daily of cetirizine or corresponding placebo, respectively. The sequence of active treatment vs. placebo were randomized. A washout period of 2 weeks was interposed between the two skin challenges. The study medication was supplied by UCB, Braine-l'Alleud, Belgium.

Microdialysis instrumentation

Two different types of microdialysis fibers were used; (1) microdialysis fibers of 216 µm outer diameter and 2 kDa molecular weight cut-off [17], and (2) fibers of 400 µm outer diameter and 3 MDa cut-off [19]. They were inserted intradermally using 21–19 gauge guide cannulae. A topical anaesthetic cream (EMLA, Astra AB, Södertälje, Sweden) was applied 45 min before the insertion. The intradermal length available for diffusion was 20 mm and the outlet length was 30 mm for both types of fibers. Individual dialysis fibers were perfused with isotonic Krebs-Ringer bicarbonate (KRB) by microinjection pumps (CMA/100, CMA/Microdialysis, Solna, Sweden).

Four 3 MDa fibers were inserted in the left forearm at 9 AM. Fibers were inserted pairwise with 2 mm between individual fibers, and 10 cm between the two series of fibers. A recovery phase of 1 h elapsed between insertion of fibers and skin challenge with allergen or buffer. Previous studies have shown no effect of local anaesthesia on histamine release from mast cell [17]. Skin challenge was performed at 10 AM, and samples from each series of fibers were collected at 1 h intervals for 7 h after skin challenge and pooled into one vial to increase sampling volume. The perfusion rate was 4 µl/min.

Four 2 kDa fibers were inserted in parallel with 4–5 cm between individual fibers in the right forearm for dose-response analysis of early allergen-induced histamine release. The insertion took place at 10 AM, immediately after skin challenge in the left arm. After insertion of fibers, a recovery phase of three hours was allowed to elapse before allergen skin challenge. A period of two hours has shown sufficient for trauma-induced hyperaemia to resolve (< 1 h) and for normalization of reflex vasodilation (flare) reactivity [17]. The perfusion rate was 3 µl/min. Allergen skin challenge was performed at 1 PM. Dialysate was

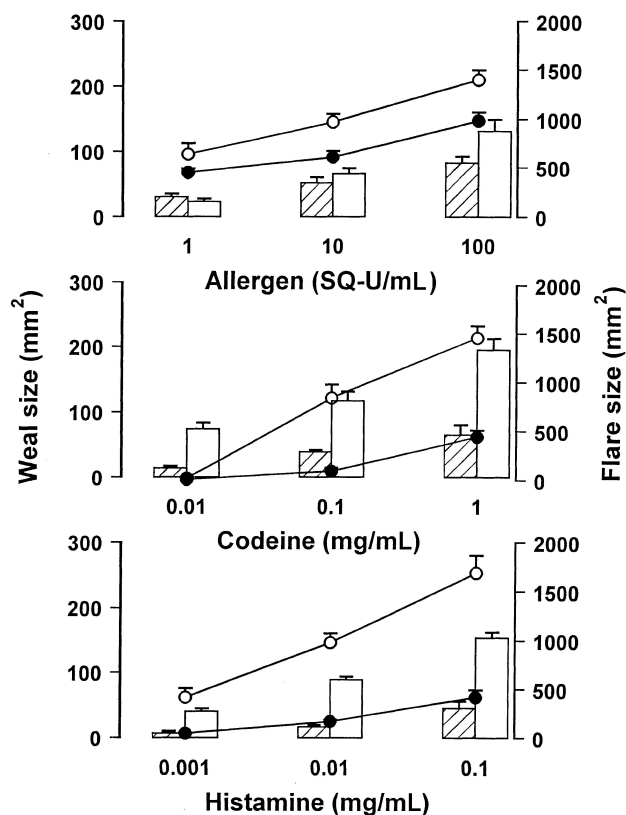


Fig. 1. Weal and flare reactions to intradermal injection of allergen (upper panel), codeine (middle panel), and histamine (lower panel) corrected for vehicle responses. In comparison to control (open bars and circles), cetirizine significantly reduced weals (solid circles, left y-axis) and flares (hatched bars, right y-axis) to all compounds. Skin reactions were read 10, 15, and 20 min after intradermal injection of histamine, codeine, and allergen, respectively. For details, see the Results section. Values are mean $\pm$ SEM ($n = 10$).

collected at 2 min intervals up to 20 min after skin challenge with allergen [18].

Skin tests

Timothy grass pollen extract (Aquagen; ALK, Copenhagen, Denmark) was diluted in ALK Diluent, a phenol-buffered solution with 0.3% human serum albumin, to working concentrations of 1–300 SQ-U/mL. Histamine (SAD, Copenhagen, Denmark) and codeine phosphate (Bispebjerg Hospital Pharmacy, Copenhagen, Denmark) were diluted in KRB to working concentrations of 0.001–0.1 mg/mL and 0.01–1 mg/mL, respectively. The two diluents were used as buffer controls. The injection volume was 25 μ L throughout the study.

Skin challenges were performed as follows: Allergen was injected intradermally above microdialysis fibers, 1 mm from the midpoint of individual dialysis fibers (right arm) or between

adjacent fibers (in the left arm). In the left forearm 300 SQ-U/mL of allergen and buffer were injected at 10 AM and dialysate was collected hourly for 7 h as described above. Immediate weal and flare reactions were not assessed. The late-phase reaction was read at 7 h (7 PM). At 1 PM, 1–100 SQ-U/mL of allergen and buffer were administered to individual fibers in the right forearm, dialysate was collected every 2 min for 20 min, and weal and flare responses read immediately thereafter.

Between 11 and 12 AM, skin reactivity to histamine (0.001–0.1 mg/mL) and codeine (0.01–1 mg/mL) was assessed in the upper arms (no microdialysis fibers inserted). Histamine-induced weals and flares were recorded after 10 min, and codeine-induced skin reactions after 15 min, respectively.

Skin reactions were outlined by ink and transferred to the subject's file for subsequent calculation of areas by a scanning device [21].

Analysis of mediators

Histamine was analysed using a glass-fiber based spectrofluorometric assay as previously described [18]. The sensitivity of this assay was 25 nM of histamine. Tryptase was assayed by fluoro-enzyme immunoassay (Pharmacia, final sensitivity after dilution of dialysate 4.0 ng/mL), PGD₂ by radio immunoassay (Amersham, Buckinghamshire, UK, 62 pg/mL), LTB₄ by enzyme immunoassay (Amersham, 6.2 pg/mL), eosinophilic cationic protein (ECP) by fluoro immunoassay, Pharmacia (8 ng/mL), myeloperoxidase (MPO) by radio immunoassay (Pharmacia, 16 ng/mL), and total protein by a photometric analysis (Coomassie assay, MRX reader, Dynatech, Eningen, Germany, 0.05 mg/mL).

Statistical analysis

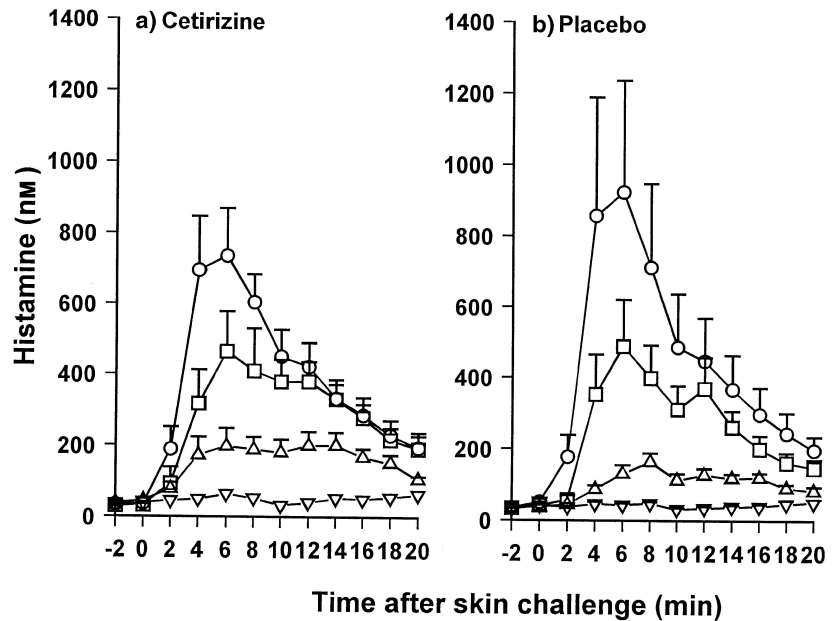
Significant changes in mediator levels over time induced by allergen were analysed using one-way repeated measurements of analysis of variance. Friedman non-parametric ANOVA was used when the test for normality failed. To test the null hypothesis of no treatment effect, a method recommended by Hill & Armitage was used [22]. The period differences were ranked and analysed using Wilcoxon-Mann-Whitney *U*-test for differences between the two sequence groups. There were no period or carry-over effects. Two-sided *P*-values < 0.05 were considered statistically significant.

Results

Immediate allergic skin responses

Intradermal injection of allergen, codeine, and histamine elicited dose-related weal and flare reactions which were potently reduced by cetirizine (Fig. 1). Cetirizine significantly

Fig. 2. Histamine release in 2 kDa fibers in skin by intradermal injection of allergen. Allergen induced a dose-related histamine release with maximum at 6–8 min. Cetirizine (panel a, left) did not significantly reduce neither the magnitude nor the kinetics of histamine release at any concentration of allergen in comparison to corresponding placebo (panel b, right). Legends: (○) 100 SQ-U/mL, (□) 10 SQ-U/mL, (△) 1 SQ-U/mL, and (▽) vehicle. Values are mean $\pm$ SEM ($n = 10$).



reduced flare reactions to all concentrations of histamine and allergen, and 0.1–1 mg/mL of codeine. In comparison to placebo, weal reactions were significantly lower on cetirizine for all concentrations of histamine and for the two largest concentrations of allergen and codeine.

Immediate allergen-induced histamine release

Intradermal injection of allergen 1–100 SQ-U/mL induced

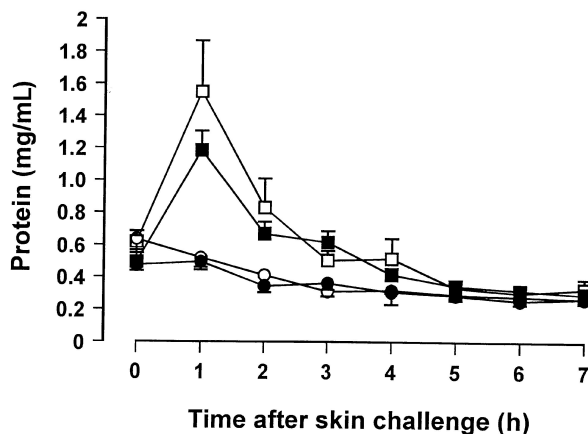


Fig. 3. Protein extravasation in allergen and buffer sites (3 MDa fibers). Allergen (300 SQ-U/mL), but not the vehicle, induced significant protein release. Cetirizine reduced protein extravasation by 40% but this did not reach statistical significance. Legends: (□) allergen response on placebo, (■) allergen response on cetirizine, (○) vehicle response on placebo, and (●) vehicle response on cetirizine.

a dose-related histamine release in 2 kDa fibers with maximum histamine levels at 6–8 min. Cetirizine did not significantly reduce peak histamine release or cumulative histamine release over 20 min (Fig. 2). The kinetics of histamine release were similar on active treatment vs. placebo.

Immediate and late-phase allergen-induced reactions

Injection of allergen 300 SQ-U/mL revealed a significant increase in dialysate protein in 3 MDa fibers with a peak concentration after one hour (Fig. 3). No plasma extravasation was observed at buffer sites. Cetirizine decreased dialysate protein levels by approximately 40% in comparison to placebo, but this difference did not reach statistical significance. No assessment of early weal and flare reactions were made for comparison.

Significant increases in histamine, tryptase, and prostaglandin D_2 (PGD_2) levels were demonstrated within the first hour in allergen sites, but not in buffer sites (Fig. 4). Cetirizine did not significantly reduce any of these mediators. In fact, PGD_2 release was significantly higher on cetirizine than on placebo. All mediators returned to baseline values within 3 h.

ECP increased significantly in allergen sites but not in buffer sites (Fig. 5). ECP levels were consistently below the detection limit of the assay in buffer sites at all measurements, but increased in allergen sites at 3 h. No significant release of MPO and LTB_4 were observed. There was no significant differences between the late-phase mediator release on cetirizine vs. placebo treatment.

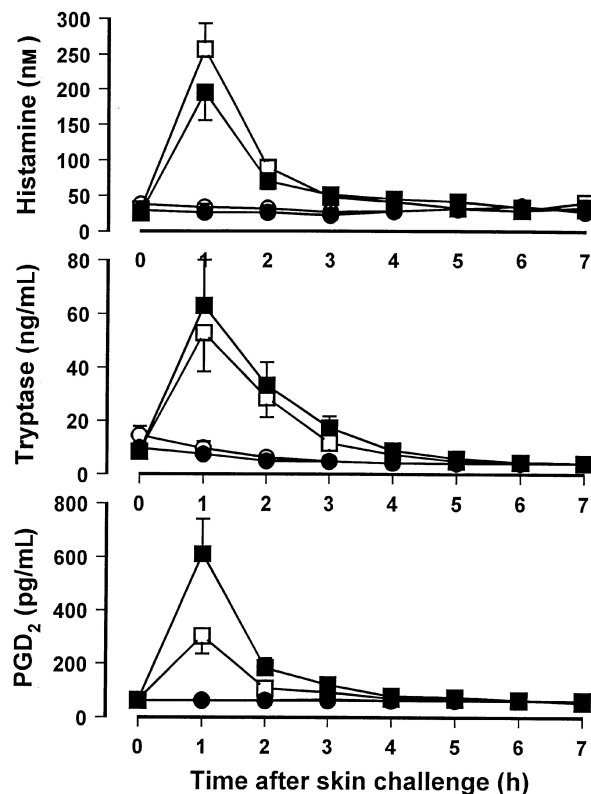


Fig. 4. Release of histamine, tryptase and PGD₂ in human skin (3 MDa fibers). A significant release of all three mediators was observed in allergen (300 SQ-U/mL) sites, but not in buffer sites. Cetirizine did not reduce histamine and tryptase levels. A significantly higher release of PGD₂ was observed on cetirizine in comparison to placebo. Legends: (□) allergen response on placebo, (■) allergen response on cetirizine, (○) vehicle response on placebo, and (●) vehicle response on cetirizine. Values are mean ± SEM (*n* = 10).

The size of the late-phase reaction was $2663 \pm 238 \text{ mm}^2$ on placebo. The reaction was significantly reduced to $1934 \pm 370 \text{ mm}^2$ by cetirizine.

Discussion

In this placebo-controlled, cross-over study we evaluated the anti-allergic effects of steady-state administration of cetirizine in daily recommended doses [1]. The results demonstrated cetirizine to be a potent H₁-receptor antagonist in the skin without any inhibitory effects on mediator release during neither the early nor the late allergic responses. In general, the findings are in contrast to data obtained in the majority of studies using skin window and skin chamber methods, but in line with data obtained by skin biopsies. The study extends a previous microdialysis study showing no effect of cetirizine, given 4 h before skin

challenge, on codeine-induced histamine release in human skin [7].

The kinetics of histamine release were described in detail, and it was similar to findings previously described by microdialysis technique [17]. Release of the mast cell mediators, histamine, tryptase and PGD₂ showed a very similar time course. Studies on the time course of mediator release in skin chamber experiments have reported a delay of hours between peak height of these mediators [5,6,23]. It cannot be excluded that variations in diffusion velocity for different mediators within the dermis to the skin chamber site at the surface of the skin as well as the low time resolution may explain variation in mediator peak levels. Recovering the mediators within the skin we were unable to observe any inhibition of early mediator release by cetirizine. At the same time, the overlying skin responses were significantly reduced. The capacity of the microdialysis technique to detect pharmacologically induced inhibition of histamine release and PGD₂ synthesis has previously been demonstrated [24]. The present study, as well as the latter one [24] included 10 subjects in each treatment arm. Based upon a prestudy power calculation (type I error 5%, type II error 10%, and a coefficient of variation of 20% on histamine release [17]), the number of patients should allow us to detect at least a 30% reduction in histamine release.

Injection of a high concentration of allergen induced an early plasma extravasation which was not significantly reduced by cetirizine. No simultaneous comparison to weal reactions were made. The lack of significant inhibition of protein extravasation during the first hour of perfusion may be due to the sampling frequency which was 1 h. Recent studies have demonstrated cetirizine to inhibit significantly the very early (approximately 10–20 min) protein extravasation induced by codeine or compound 48/80 without any effect on late (60 min) reaction [25].

Until recently mainly low molecular weight mediators have been measured in microdialysis experiments. Recent innovations have permitted sampling of high molecular weight compounds with microdialysis technique, e.g. total protein and tryptase [19,20]. In this study the time courses of mediator levels from cells suspected to play pivotal parts in the developing late-phase reactions were described.

Increase of ECP was evident from 3 h and onwards, and this response was not inhibited by cetirizine. Histological studies have demonstrated an influx of eosinophils into the dermis at 3 h [26] and no effect of cetirizine on eosinophil influx at later time points [13,14,16]. No significant release of MPO was evident, and the eosinophil chemoattractant LTB₄ did not increase significantly over 7 h. The latter finding is in agreement with findings by Kuhns and coworkers [27]. No late histamine release was observed. Activated basophils have been suggested to cause release of

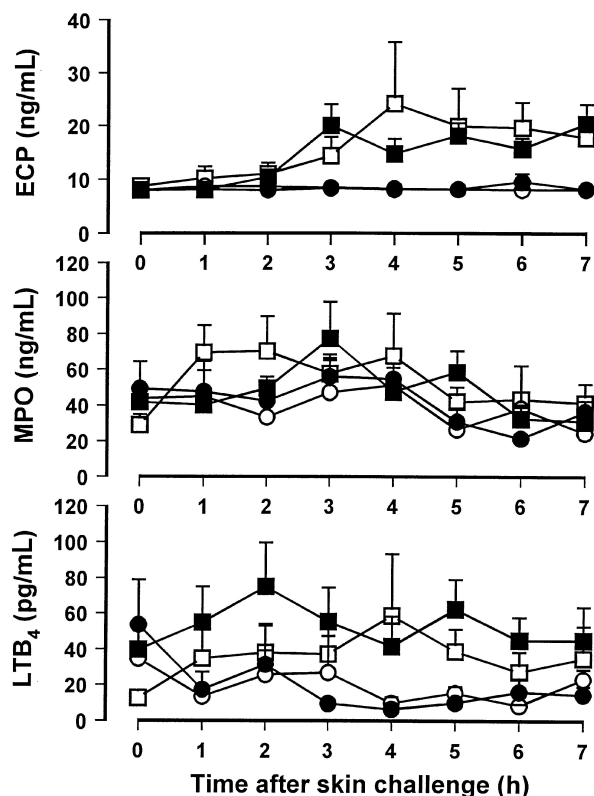


Fig. 5. Release of ECP, MPO and LTB₄ in human skin (3 MDA fibers). A significant release of ECP was observed after intradermal injection of allergen (300 SQ-U/mL), but not buffer. Cetirizine did not reduce this response. Neither MPO nor LTB₄ increased significantly following skin challenge with allergen or placebo. Legends: (□) allergen response on placebo, (■) allergen response on cetirizine, (○) vehicle response on placebo, and (●) vehicle response on cetirizine. Values are mean $\pm$ SEM ($n = 10$).

histamine hours after the initial allergen challenge. However, previous studies have shown that a significant influx of basophils and release of histamine do not occur until 7–8 h [28,29].

Cetirizine inhibited significantly the late-phase infiltration at 7 h without any impact on the mediators analysed. In most of the previous studies cetirizine inhibited the late-phase reaction by approximately 20–40%, the results being statistically significant in some of the studies [6,28]. Similar observations have been demonstrated with terfenadine which was without effect on mediator release in human skin *in vivo* as well as *in vitro* [23].

The pivotal role of eosinophils in the allergic late-phase reaction has been debated for years [30]. Our findings indicate no clear relationship between late-phase reaction (LPR) and eosinophil activity. These data are in line with recent studies showing the β_2 -agonist salmeterol to inhibit allergen-induced late-phase reactions without inhibition of

total cellular influx or eosinophil recruitment [31] or ECP and MPO levels in suction blister fluid [24]. The pathophysiology of the late-phase allergic response is complex and many other cells and mediators may be involved than those actually measured. Further insight into the inflammatory mechanisms of the human skin is requested.

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