

Effect of cetirizine and prednisolone on cellular infiltration and cytokine mRNA expression during allergen-induced late cutaneous responses

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Summary

Background The ability of cetirizine to inhibit eosinophil infiltration into the sites of allergen-induced cutaneous late-phase reactions is controversial. A previous skin biopsy study gave negative results with 15 mg of cetirizine as a single dose.

Objective To confirm these findings we have used cetirizine (30 mg daily) for 5 days and compared the results with prednisolone (20 mg daily for 5 days) as a positive control. The effect of these agents on mRNA positive cells for interleukin-3 (IL-3), interleukin-4, interleukin-5 and granulocyte/macrophage-colony stimulating factor (GM-CSF) was also evaluated.

Methods A double-blind, placebo-controlled cross-over study ($n = 12$) was followed. After each treatment 30 biological units (BUs) of *Dermatophagoides pteronyssinus* or *Phleum pratense* were injected intradermally and the early (15 min) and late-phase response sizes (6 and 24 h) were measured. Skin biopsies were taken at 24 h for immunocytochemistry and *in situ* hybridization.

Results Cetirizine but not prednisolone inhibited the early-phase response (37%, $P = 0.004$). In contrast prednisolone, but not cetirizine, significantly inhibited the size of the late-phase reaction at 24 h (70%, $P = 0.021$). This was associated with significant decreases in total (MBP+) and activated (EG2+) eosinophils ($P = 0.019$ and 0.014 , respectively), as compared with placebo. There were also clear but non-significant reductions in interleukin-3, interleukin-4, interleukin-5 and granulocyte/macrophage-colony stimulating factor mRNA+ cells.

Conclusion Prednisolone, but not cetirizine, inhibited both the magnitude of the allergen-induced late-phase response and the accompanying local eosinophil infiltration. These corticosteroid effects were associated with a reduction in cells expressing mRNA for 'TH₂-type' cytokines.

Keywords: cetirizine, late-phase response, prednisolone, eosinophil, TH₂ cytokine, interleukin-4, interleukin-5, interleukin-3, granulocyte/macrophage-colony stimulating factor

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Introduction

Cetirizine is a non-sedating selective H₁ histamine antagonist, which inhibits the cutaneous early-phase

reaction (EPR) [1,2] and is clinically effective in the treatment of both seasonal and perennial rhinitis and chronic idiopathic urticaria [3]. By use of the skin window technique, several studies have shown that cetirizine reduced eosinophil influx into the late-phase reaction (LPR) [4,5]. In a previous double-blind, placebo-controlled cross-over study involving skin

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biopsies of cutaneous LPR and the administration of single doses of prednisolone (20 mg) and cetirizine (15 mg), we observed only a slight, non-significant decrease in eosinophil infiltration (MBP+ cells) and activation (EG2+ cells) by cetirizine [1]. This has prompted us to re-evaluate the actions of cetirizine on cell infiltration during the LPR using higher doses (30 mg daily) for a more prolonged period (5 days). In addition we have examined the effects of prednisolone and cetirizine on the cytokine pattern (IL-3, IL-4, IL-5 and GM-CSF) associated with the allergen-induced LPR.

As in the previous study we have compared cetirizine with prednisolone. Corticosteroids inhibit the late-phase response through actions that are not very clear [1,6,7]. *In vitro* and *in vivo* studies suggest that the principal cellular targets of steroid action may be T lymphocytes, eosinophils and/or neutrophils [1,8–10]. Corticosteroids inhibit production of T cell-derived cytokines both *in vivo* and *in vitro* [11–13] and are associated with a decrease in the numbers of T cells and activated eosinophils during the LPR [1].

In atopic asthma corticosteroids suppress the accumulation in mRNA+ cells for TH₂-type cytokines [14]. For these reasons we have also performed *in situ* hybridization for IL-3, IL-4, IL-5 and GM-CSF transcripts.

Materials and methods

Patients

Twelve atopic subjects (age range 19–48 years) were selected from the Allergy Clinic of the Royal Brompton Hospital. They all had a history of perennial or seasonal allergic asthma and/or rhinitis, a positive skin-prick test >5 mm to house dust mite or grass pollen extract (Bencard Ltd, Brentford, Middlesex, UK), and a 6 h cutaneous late-phase reaction of >30 mm to 30 BU of intradermal timothy grass pollen (*Phleum pratense*) or house dust mite (*Dermaphagoides pteronyssinus*) (Aquagen SQ, ALK, Horsholm, Denmark). Two other patients who had fulfilled entry criteria were excluded from the study on grounds of safety since they had moderate systemic reactions after intradermal allergen challenge (see results).

Patients were excluded if they had other medical diseases, known impairment of liver, kidney or cardiac function, were pregnant or breast-feeding, were taking regular oral medication, had undergone recent (<2 years) injection immunotherapy or had a history of allergy to hydroxyzine. Subjects that could not suspend oral medication during the preceding period with astemizole (3 months), ketotifen and hydroxyzine (1 month), corticosteroids (3 weeks), cromoglycate and sodium

nedocromil (2 weeks), non-steroidal anti-inflammatory drugs, other antihistamines or anti-allergic drugs (48 h) were also excluded.

Study design

The study was performed with the approval of the Royal Brompton Hospital Ethics Committee and all patients gave their written informed consent. The design was a double-blind, double-dummy, placebo-controlled cross-over study of 5-day treatments with cetirizine, prednisolone and placebo. The sequence of administration of the study medications was randomized in a four independent Latin square design in blocks of three.

Drug treatment regimen

The study drugs were supplied by UCB (Braine l'Alleud, Belgium). The dose of prednisolone administered was 20 mg, once daily for 5 days, and cetirizine 15 mg twice daily for 5 days. In order to maintain 'blinding', placebo tablets were used to make all treatment packs identical and consisting of seven tablets daily. Between each challenge, a minimum period of 3 weeks was allowed in order to eliminate any carry-over effect. Patients challenged with grass pollen were studied outside the grass pollen season. Subjects were instructed to suspend any oral medication (antihistamines, corticosteroids, sodium cromoglycate, nedocromil, non-steroidal anti-inflammatory drugs, ketotifen) before the cutaneous challenge and biopsy.

Allergen extracts

Lyophilized extracts of timothy grass pollen or *Dermaphagoides pteronyssinus* were obtained from ALK Denmark (Aquagen SQ) and reconstituted at 1500 BU/mL in isotonic sterile saline. A single batch of diluted allergen was aliquoted and stored at –20°C.

Skin testing

All intradermal skin tests were performed in the morning between 08.00 h and 10.00 h. Skin tests were performed in duplicate, 10 cm apart on the extensor aspect of the forearm. Allergen (30 BU) (house dust mite or timothy grass pollen) in 0.02 mL of saline was injected at each site. The reaction sizes were measured in two dimensions at 15 min, 6 h and 24 h by detecting cutaneous induration by resistance to the movement of a sharpened pencil point. The indurated area was then outlined by a marker and a permanent sticky tape record of the 6 and 24 h reactions was taken. The extensor aspect of the forearm

was chosen to reduce visibility of the biopsy scar. Skin tests and biopsies for each drug challenge were performed on alternate forearms commencing with the left.

Skin biopsies and tissue processing

A 3-mm disposable punch biopsy was used. Following each course of oral treatment, a single biopsy was taken at 24 h using 1% plain lignocaine as local anaesthesia. Each biopsy was divided and each half processed separately for immunohistology and for *in situ* hybridization studies. Biopsies for immunohistology were immediately placed in PBS and snap-frozen in OCT embedding medium using isopentane, precooled in liquid nitrogen. Biopsies were then stored at -80°C . Six micrometre sections were cut on a cryostat (Bright Instruments, Huntingdon, UK) and consecutive sections were placed on microscope slides. They were air dried for 1 h, fixed in 60/40 acetone/methanol for 5 min, and further air dried for 1 h. The slides were then wrapped in aluminium foil and stored at -80°C before immunostaining.

Biopsies for *in situ* hybridization were immediately placed in 4% paraformaldehyde in 0.1 M PBS for 1 h, followed by 15% sucrose/PBS for 1 h, and then subsequently in 15% sucrose/PBS overnight. Biopsies were then placed in OCT embedding medium, snap frozen in isopentane precooled in liquid nitrogen, and stored at -80°C . Some 10 μm cryostat sections were cut, and single sections were placed on poly-L-lysine coated microscope slides and then baked overnight at 37°C . Slides were then stored at -80°C in the presence of silica gel.

Immunohistology

Monoclonal antibody staining was detected by the alkaline phosphatase anti-alkaline phosphatase method, as described [15]. Normal human serum (20%) was used to prevent non-specific binding of the second and third layer antibodies. Sections of human tonsil obtained at routine tonsillectomy operations were included as positive controls in each staining run. A mouse IgG1 myeloma protein was used as a negative control. The monoclonal antibodies used were: CD45, CD3, CD4, CD25 (Becton Dickinson, Cowley, Oxford, UK); EG2, a monoclonal antibody that recognizes the cleaved, actively secreted form of eosinophil cationic protein (ECP) (Pharmacia, Milton Keynes, UK); CD8 and CD68 (macrophage) (DAKOPATTS, High Wycombe, UK); anti-elastase (neutrophil). Polyclonal rabbit anti-mouse immunoglobulin and APAAP reagents were purchased from DAKOPATTS. Optimal concentrations for immunostaining for each antibody were determined so

that discrete staining of individual cells was achieved with a low background. All other chemicals and solvents for immunohistology were purchased from BDH (Dagenham, UK). For each monoclonal antibody, staining of all sections was completed in one experiment using the same batch of antibodies.

In situ hybridization

All reagents were from Sigma Chemicals (Poole, UK) unless otherwise indicated. Riboprobes labeled with ^{35}S were prepared from cDNAs for IL-3, IL-4, IL-5 and GM-CSF (Glaxo Biogen, Geneva, Switzerland), as previously described [15]. Incubation in *n*-ethylmaleimide iodoacetamide, and triethanolamine reduced non-specific binding of the ^{35}S probes. Negative controls employed were the sense probe (identical nucleotide sequence to mRNA) and pretreatment of slides with ribonuclease A (Promega, Southampton, UK). The positive control was a PHA-stimulated T-cell clone known to produce IL-2, IL-4, IL-5 and GM-CSF. Cells expressing cytokine mRNA were detected as discrete, dense deposits of silver grains on autoradiographs. Owing to a power failure involving a -80°C freezer, some sections from some of the biopsies for *in situ* hybridization studies were destroyed. This resulted in sections from only seven to nine patients being available for study and in the need for repeating some of the biopsies in some patients.

Quantitation

Slides were coded and counted blind using a BH2 microscope (Olympus Corp., Lake Success, NY, USA) at a magnification of $200\times$ with an eyepiece graticule. For immunohistology, at least four sections from each biopsy were stained with each monoclonal antibody and a total of three to six grids per section were counted. The upper edge of the grid was placed at the dermal/epidermal junction. In areas containing sweat ducts and hair shafts, only intervening dermal regions were counted. The results were expressed as mean positive cells per field (0.202 mm^2). Cells expressing positive hybridization signals for cytokine mRNA were counted in a similar fashion. The within observer coefficient of variation for repeat measurements for immunocytochemistry and *in situ* hybridization was less than 5%.

Analysis

Data were analysed using two statistical packages (Minitab software; Minitab Inc., University Park, PA, USA, and SAS software, SAS Institute, Carey, NC, USA). Mean surface areas of the allergen-induced early and

late-phase cutaneous responses after prednisolone or cetirizine treatment were compared with those obtained after placebo using Wilcoxon signed rank test. Within individual subjects, cell counts for phenotype and cytokine mRNA after allergen were compared with the corresponding counts after diluent using the Wilcoxon matched pairs signed rank test. Between group comparisons of changes in cell counts after challenge (allergen minus diluent) were performed by Friedman's test for the three treatments. Results were then analysed in a two by two comparison, using Wilcoxon's signed rank test. Regression analysis was performed with Spearman's test. *P*-values less than 0.05 were considered significant.

Results

Early (EPR) and late (LPR) cutaneous responses

Systemic reactions occurred in two subjects after intradermal allergen challenge. In one case, the patient developed chest tightness and wheezing 20 min after intradermal injection of the allergen. This patient gave a history of mild asthma but was asymptomatic at the time of testing. A second, non-asthmatic patient developed a reaction at 15 min, consisting of urticarial rash on the hands, flushing, 'tightness' in the throat and periorbital and facial swelling. Both reactions responded promptly to the subcutaneous administration of adrenaline and intravenous administration of methylprednisolone or hydrocortisone. These patients were subsequently excluded from the study.

The effect of 5 days treatment with prednisolone (20 mg once daily) and cetirizine (15 mg twice daily) on the size of the allergen-induced cutaneous responses are shown in Table 1. Cetirizine decreased the 15 min EPR weal surface area by 37% ($P = 0.004$; Wilcoxon signed rank test) compared with placebo and showed a trend for reduction of the magnitude of the LPR ($P = 0.083$) at 6 h but not at 24 h. Prednisolone treatment did not significantly alter the EPR but produced a decrease in the size of the LPR at 6 h which reached significance ($P = 0.021$) at 24 h.

Immunohistology

When compared with diluent-challenged sites, there were significant increases ($P < 0.05$; Wilcoxon signed rank test) in practically all phenotypic or activation markers studied at allergen sites in placebo, cetirizine and prednisolone-treated groups.

Median changes in cell counts after allergen challenge (allergen minus diluent) were compared between prednisolone, cetirizine and placebo treatments and showed that prednisolone significantly inhibited the allergen-induced increase in MBP+ and EG2+ cells ($P = 0.019$ and $P = 0.014$, respectively) (Table 2). Individual values for MBP and EG2 are shown in Fig. 1. In addition, prednisolone reduced the median number of infiltrating neutrophils by 50%, although this effect did not reach statistical significance. With cetirizine there were no significant changes in cell numbers or activation markers for any cell type, although compared to placebo the

Table 1. Effects of prednisolone, placebo and cetirizine on the magnitude (mean weal surface area) of the allergen-induced cutaneous early and late-phase responses ($n = 12$)

Treatment	Median weal surface area (mm ²)	Range (min-max)	Inhibition from placebo (%)	<i>P</i> -value
<i>15 min skin reaction</i>				
Placebo	347	[280-994]		
Cetirizine	217	[118-434]	37	0.004*
Prednisolone	360	[221-1502]	1	0.248
<i>6 h skin reaction</i>				
Placebo	1869	[814-3972]		
Cetirizine	1582	[395-3680]	15	0.083
Prednisolone	1498	[285-2538]	20	0.083
<i>24 h skin reaction</i>				
Placebo	3123	[826-4773]		
Cetirizine	2235	[0-7110]	28	0.248
Prednisolone	948	[1-3484]	70	0.021*

P-values shown were obtained with Wilcoxon signed rank test.

Table 2. Immunohistology of cutaneous late-phase reaction after 5 days of treatment with prednisolone, cetirizine or placebo ($n = 12$)

	Placebo			Prednisolone			Cetirizine			Friedman's test <i>P</i> -values
	Control (median)	Allergen (median)	Difference (range)	Control (median)	Allergen (median)	Difference (range)	Control (median)	Allergen (median)	Difference (range)	
CD45	11.9	47.5	29.4 (-4.4, 56.8)	12.5	46.0	35.5 (2.5, 60.9)	22.8	49.9	30.9 (-5.6, 91.9)	0.44
CD3	4.9	27.4	17.5 (-4.1, 47.7)	4.5	26.1	17.7 (-3.5, 49.7)	7.3	28.0	13.3 (-4.1, 126.0)	0.49
CD4	7.4	32.6	14.0 (-12.1, 87.4)	8.3	20.7	12.8 (-5.1, 59.8)	7.9	18.6	12.2 (-14.2, 73.2)	0.91
CD8	1.5	4.8	2.3 (-1.0, 14.4)	1.7	5.8	3.1 (-0.7, 8.0)	1.6	3.4	1.3 (-1.9, 13.4)	0.25
MBP	0.0	14.0	13.0 (-1.5, 30.8)	0.0	2.6	2.6 (-0.2, 18.3)	0.5	17.1	16.1 (2.2, 25.1)	0.04*
EG2	0.0	7.9	7.9 (-1.5, 32.4)	0.0	3.1	3.1 (-1.1, 6.6)	0.3	9.9	9.1 (0.7, 24.6)	0.09**
CD25	1.1	2.2	0.6 (-0.6, 6.8)	0.3	3.6	1.9 (-3.0, 5.0)	2.1	2.2	1.4 (-3.7, 8.7)	0.74
HLA-DR	37.0	86.4	24.5 (-20.1, 82.4)	34.5	53.8	25.7 (-26.3, 71.9)	50.5	63.4	20.0 (-39.7, 59.8)	0.20
NE	1.0	16.3	12.7 (-9.5, 26.1)	0.9	7.9	6.3 (-8.9, 48.2)	0.5	16.6	6.5 (-1.7, 21.2)	0.74

*Wilcoxon test (Pred-Plac) $P = 0.02$. **Wilcoxon test (Pred-Plac) $P = 0.01$. Median cell counts are shown for each monoclonal antibody together with median differences and 95% confidence intervals. Significant or almost significant P values with asterisks were followed by analysis with Wilcoxon signed rank test. CI. Confidence interval.

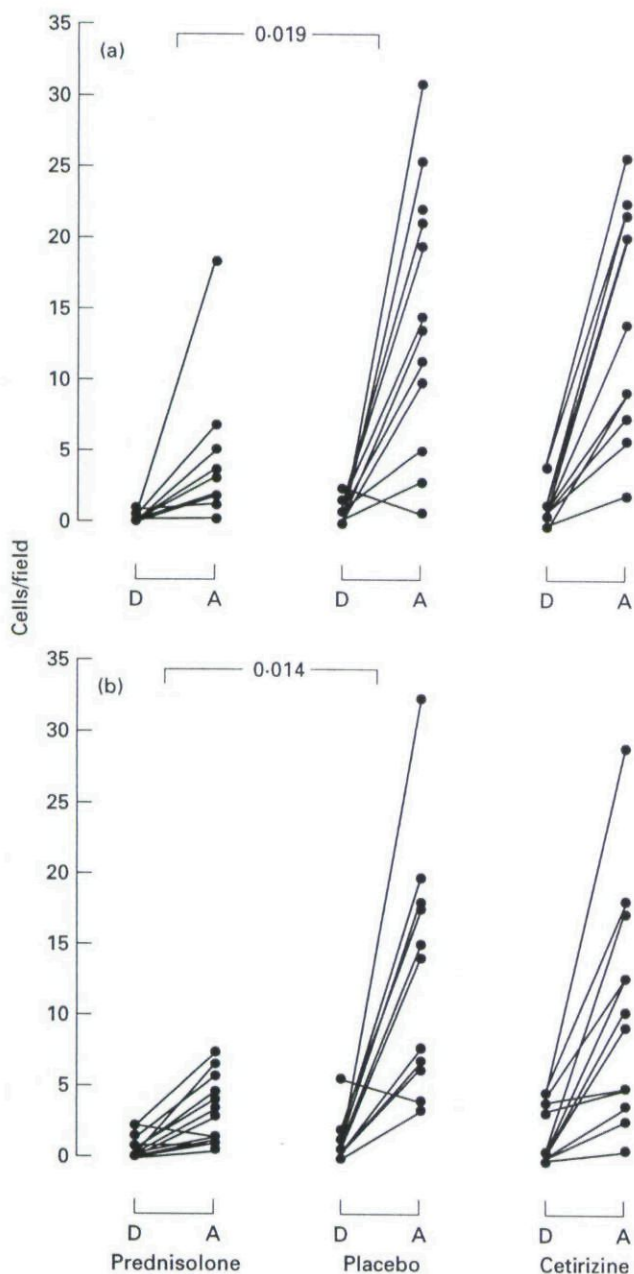


Fig. 1. Individual cell counts for expression of (a) MBP and (b) EG2 after treatment with prednisolone, cetirizine or placebo. *P*-values shown were calculated with Wilcoxon signed rank test. D. Diluent. A. Allergen.

magnitude of the allergen-induced increases in MBP+ and EG2+ cells were reduced in five patients out of 12 tested.

In situ hybridization

When compared with diluent sites, there were significant increases in IL-3, IL-4, IL-5 and GM-CSF expression at

allergen sites in placebo, cetirizine and prednisolone-treated groups.

The effects of prednisolone on cytokine mRNA in the 24 h LPR are shown in Table 3. Median changes in cell counts after allergen challenge (allergen minus diluent) were compared between prednisolone, cetirizine and placebo treatments and revealed that prednisolone showed a trend for decreasing expression of mRNA for IL-4 (85% reduction in median cell numbers; $P = 0.063$; Friedman's test) and was associated with a reduction in the median numbers of cells expressing mRNA for IL-3, IL-5 and GM-CSF by 70%, 50% and 63%, respectively. When compared with placebo, prednisolone decreased the number of allergen-induced IL-4 mRNA+ cells ($P = 0.06$; Wilcoxon test) and was also associated with a decrease in GM-CSF, IL-3 and IL-5 mRNA+ cells when compared with cetirizine ($P = 0.004$, $P = 0.08$ and $P = 0.06$, respectively; Wilcoxon test). Cetirizine was also associated with a reduction in IL-4 mRNA+ cells (57% decrease), although this was not significant. Individual values for IL-3, IL-4, IL-5 and GM-CSF are shown in Fig. 2.

Correlation between MBP and EG2 numbers and IL-5 mRNA+ cells

In view of the decreases in TH₂-type cytokines with prednisolone treatment, we correlated the magnitude of these decreases with the decrease in MBP+ cells with the same treatment. The magnitude of the decrease (prednisolone vs placebo) in the numbers of MBP+ cells was found to be positively correlated with the decrease (prednisolone vs placebo) in the numbers of IL-5 mRNA+ cells, after allergen challenge (Spearman's test, $r = 0.721$; $P = 0.028$).

Discussion

In this report we show that 5 days of treatment with prednisolone but not cetirizine inhibited the cutaneous LPR at 24 h and that this was accompanied by a local decrease in the number of total and activated eosinophils in response to allergen challenge. We have further shown that prednisolone treatment was also associated with a clear decrease in IL-3, IL-4, IL-5 and GM-CSF mRNA positive cells during the LPR. These findings suggest that corticosteroids may inhibit the cutaneous LPR and associated eosinophilia at least in part by inhibiting cytokine gene expression. We have also shown that cetirizine treatment significantly inhibited the EPR and that an inhibitory effect was still observable (although not significant) 6 h after allergen challenge. Cetirizine treatment was also associated

Table 3. *In situ* hybridization of cutaneous late-phase reaction after 5 days of treatment with prednisolone, cetirizine or placebo ($n = 9$)

	Placebo			Prednisolone			Cetirizine			Friedman's test <i>P</i> -values
	Control (median)	Allergen (median)	Difference (range)	Control (median)	Allergen (median)	Difference (range)	Control (median)	Allergen (median)	Difference (range)	
IL-3	0.3	9.0	7.1 (-0.2, 21.1)	0.3	2.7	2.0 (0.0, 7.5)	0.8	6.4	5.3 (0.5, 42.7)	0.19
IL-4	0.9	13.2	12.5 (-0.8, 23.0)	0.0	2.0	3.8 (-2.0, 9.6)	0.2	5.7	6.8 (0.0, 33.7)	0.047*
IL-5	1.0	10.6	8.5 (0.0, 19.4)	1.5	5.2	1.3 (-1.1, 14.1)	2.0	8.7	6.7 (0.7, 27.4)	0.21
GM-CSF	0.7	11.8	8.3 (0.0, 28.4)	0.1	4.4	4.1 (-1.1, 8.5)	0.4	10.2	16.0 (3.3, 29.3)	0.17

*Wilcoxon test (Pred-Plac) $P = 0.06$. Median cell counts are shown for each cytokine mRNA probe together with median differences and 95% confidence intervals. *P*-values were obtained with Friedman's test. CI. Confidence interval.

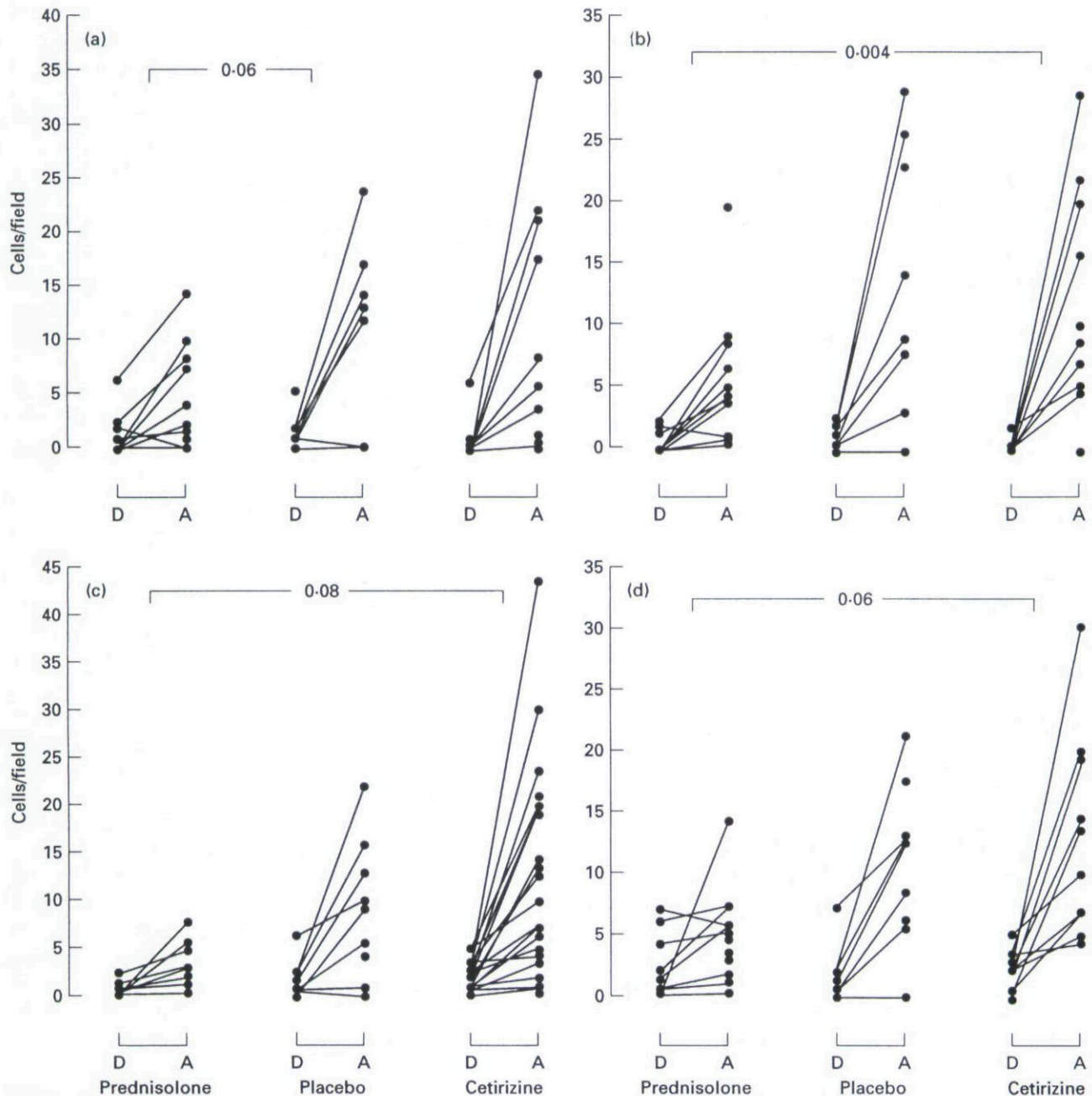


Fig. 2. Individual cell counts for expression of mRNA for (a) IL-4, (b) GM-CSF, (c) IL-3 and (d) IL-5 after treatment with prednisolone, cetirizine or placebo. *P*-values shown were calculated with Wilcoxon signed rank test. D. Diluent. A. Allergen.

with a moderate decrease in IL-4 mRNA + cells during the LPR.

We performed a double-blind, placebo-controlled cross-over study where each subject acted as his own control. Two patients developed immediate systemic reactions occurring at 15 and 20 min after intradermal allergen injection and were excluded from the study. One of these reactions occurred in a patient with mild asthma

and the other in a non-asthmatic subject. We do not routinely exclude patients with asthma, since reactions are rare and in our experience no more likely to occur in asthmatic patients after intradermal challenge. However, the possibility of systemic reactions occurring stresses the importance of performing intradermal challenges in a well-equipped room, under the supervision of experienced staff and highlights the need to keep patients under

observation for at least 1 h after intradermal allergen injection. We used relatively high doses of cetirizine (30 mg daily) and moderate doses of prednisolone (20 mg daily) for a period of 5 days, and allowed an adequate interval between each treatment to avoid any carry over effect. Skin biopsies were taken and processed for immunohistology and *in situ* hybridization following well established protocols that have been previously used by us to study cellular and molecular aspects of allergic inflammation in the skin, nose and lung [15–20]. Although some biopsy sections were lost due to a freezer power failure we nonetheless obtained significant or almost significant results for prednisolone both in terms of cellular infiltration and cytokine mRNA expression.

The LPR is a phenomenon that depends on specific IgE [21,22], through activation of mast cells with release of preformed and *de novo* mediators but which also involves a more complex network of both humoral and cellular factors (T lymphocytes, eosinophils, basophils) where cytokines may have an important role by attracting, activating and altering functional properties of cells [23–28]. This report confirms that allergen challenge induces a significant increase in inflammatory cells, namely T lymphocytes, eosinophils and neutrophils that parallels the macroscopic response and that is accompanied by an increase in cells positive for IL-3, IL-4, IL-5 and GM-CSF mRNA. Corticosteroids have been shown to inhibit the cutaneous LPR [1,6,7] through mechanisms that are not well known, but that may include reduced neutrophil, monocyte and eosinophil infiltration and activation [1,8]. We have shown in this study that prednisolone treatment but not cetirizine is associated with a clear inhibition of both the cellular infiltrate and cytokine mRNA expression during the LPR. There was a highly significant decrease in the number of infiltrating eosinophils (MBP+ cells) and, more importantly, prednisolone pretreatment also inhibited allergen-induced eosinophil activation as shown by a decrease in the number of EG2+ cells (secretory form of ECP). Prednisolone pretreatment was also associated with a reduction in the median numbers of neutrophils at 24 h. In addition, prednisolone showed a trend for inhibition of the increase in IL-4 mRNA+ cells ($P = 0.063$) and clearly reduced the numbers of IL-3, IL-5 and GM-CSF mRNA+ cell in response to allergen challenge, at 24 h. The preferential reduction in IL-4 mRNA+ cells is in line with the results obtained with the topical use of corticosteroids in the allergen-induced late nasal response, which was shown to result in a significant decrease in IL-4 but not in IL-5 mRNA positive cells [10]. On the other hand, our results are only in partial agreement with those showing that oral

prednisolone treatment in moderately severe atopic asthmatic patients was associated with a decrease in the number of baseline mRNA positive cells for IL-4 and IL-5 in the bronchoalveolar lavage fluid [14]. Differences in kinetics of cytokine mRNA expression between cells that infiltrate the nasal or bronchial LPR and those that are present in the skin during the cutaneous LPR may explain these discrepancies. Phenotypic differences have already been described and the possibility that functional differences may also be relevant should also be considered [29]. Alternatively, differences between baseline (lung study) and challenge studies (nose and skin studies) may explain the discrepancies in the prednisolone-associated decreases in IL-4 and IL-5 mRNA+ cells. We believe our results give further support to the idea that corticosteroids may inhibit the allergen-induced LPR at least in part by downregulating the expression of cytokine genes [12,13]. A decrease in IL-4 mRNA+ cells may be relevant to the inhibition of the infiltration of eosinophils observed during the LPR, since IL-4 has been shown to upregulate endothelial cell expression of VCAM-1, an important molecule for transendothelial migration of eosinophils into tissues [23]. Although a direct effect on eosinophils cannot be excluded [30], a decrease in IL-3, GM-CSF and IL-5 mRNA expression with prednisolone treatment may be relevant for both the inhibition of eosinophil infiltration and activation in the cutaneous LPR. This association is further supported by the positive correlation between the magnitude of the decrease in MBP+ cell numbers and the decrease in IL-5 mRNA positive cells after prednisolone treatment.

Cetirizine has potent antihistamine actions [2] and is reported to have anti-inflammatory properties that may include inhibition of eosinophil chemotaxis during the LPR [4]. We have confirmed that cetirizine (15 mg twice daily, for 5 days) is efficacious at inhibiting the immediate weal-and-flare reaction. Although cetirizine did not inhibit the macroscopic magnitude of the LPR at 24 h, a trend for blunting the LPR 6 h after allergen challenge was apparent.

Skin window studies have reported a significant reduction in the number of eosinophils during the LPR with cetirizine pretreatment [4,31] but we have not been able to confirm this in skin biopsies in the present and a previous report [1], although in our study cetirizine pretreatment was associated with slight to moderate decreases in MBP+ and EG2+ cells in five out of 12 patients studied. The reason for this discrepancy may be that biopsy sections evaluate the overall cellular content in the LPR but do not discriminate between cells that have undergone transendothelial migration into the skin and those that are still in the dermal vasculature. Skin windows techniques study changes in cells that have

migrated into the tissues but not those that remain in the vasculature and are therefore more sensitive to changes in transendothelial migration. Others have indeed reported that high concentrations of cetirizine can prevent adhesion of human eosinophils to vascular endothelial cells, *in vitro* [32]. Future studies, using skin windows and skin biopsies in the same patients should help clarify the reason for the discrepancies observed. An alternative explanation may be that the anti-inflammatory effect of cetirizine on cell infiltration and cytokine mRNA during the LPR, being less potent than those of prednisolone, will only be detectable in some patients. This is supported by the fact that in our study cetirizine inhibited eosinophil and IL-4 mRNA + cell infiltration in some patients, although this was not significant.

In summary, our results indicate that the decrease in the magnitude of the allergen-induced cutaneous LPR after prednisolone treatment was associated with a reduction in the numbers of total (MBP+) and activated (EG2) eosinophils and that this decrease in MBP+ and EG2+ cells may be partly due to inhibition of TH₂-type cytokine expression. In addition, our findings also confirmed that cetirizine inhibited the allergen-induced EPR and suggest that this drug also moderately decreased the magnitude of the cutaneous reaction 6 h but not 24 h after intradermal allergen challenge.

Our results suggest future targets for anti-allergic treatment. Focusing research on blockage of the actions of GM-CSF IL-5 and IL-4 in the late-phase reaction with synthesis inhibitors, soluble receptors or monoclonal antibodies (MoAb) directed against these cytokines may confirm the importance of these cytokines for allergic inflammation. For example, IL-4 deficient mice have substantially fewer eosinophils and less peribronchial inflammation as compared with the normal mice in response to bronchial challenge with a relevant allergen [33].

Furthermore, pre-treatment with anti-IL-5 MoAb inhibited antigen-induced eosinophil infiltration into the trachea and associated hyperreactivity in murine and guinea-pig models [34,35].

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