

Effects of fluticasone propionate and beclomethasone dipropionate on parameters of inflammation in peripheral blood of patients with asthma

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Bronchial inflammation plays a central role in asthma. We investigated whether parameters of inflammation were increased in peripheral blood. Furthermore, we tested whether fluticasone propionate (FP), a new inhaled corticosteroid (ICS), and beclomethasone dipropionate (BDP) affected these parameters. FP 750 µg/day and BDP 1500 µg/day were compared in a randomized, crossover study consisting of two 6-week treatment periods, each preceded by a 3-week placebo period. Twenty-one patients with symptomatic asthma completed the study. The results were compared with those of six normal subjects (controls). Immunophenotyping of inflammatory cells was performed in whole blood, and serum eosinophil cationic protein (ECP) was measured. With regard to clinical efficacy, ICS increased PC₂₀ histamine by more than 1.9 doubling doses and FEV₁ by more than 0.34 l. The number of CD3/HLA-DR+ lymphocytes was significantly increased in asthmatics compared to the normal subjects, both after placebo ($P < 0.01$) and after therapy ($P < 0.05$). The CD3/HLA-DR+ lymphocytes decreased significantly after treatment with FP ($P < 0.05$). Serum ECP was elevated in patients without ICS and decreased after treatment with BDP ($P < 0.001$). In conclusion, the number of CD3/HLA-DR+ lymphocytes and serum ECP levels were raised in the peripheral blood of symptomatic asthmatics, and decreased by clinically effective doses of ICS. In this respect, FP 750 µg/day was at least as effective as BDP 1500 µg/day.

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Bronchial inflammation plays a crucial role in the pathogenesis of asthma, and is accompanied by increased bronchial hyperresponsiveness (BHR) (1, 2). The persistent inflammation of the bronchial mucosa in asthmatic subjects, which is characterized by infiltration of eosinophils, is, at least partly, orchestrated by T cells (2, 3). Increased numbers of T cells, many of which are CD4-positive (T-helper) cells and exhibit the phenotype of inflammatory activated state, are invariably seen in the mucosa. Cytokines, such as interleukin (IL)-3, IL-5, and GM-CSF, produced by these activated T cells modulate inflammatory cells, including eosinophils (4, 5).

Signs of this inflammation can be measured in the airways by means of biopsies, and the inflammation is characterized by increased numbers of T cells expressing activation markers and activated eosinophils. These activated eosinophils release preformed mediators from their granules, such as eosinophil cationic protein (ECP), major basic protein, and eosinophil-derived neurotoxin. Bronchial inflammation is thought to be reflected in the peripheral blood by expression of activation epitopes on T cells (6–8), and elevated ECP levels present in the serum (9). These elevated ECP levels are possibly related to the severity of asthma (9).

Inhaled corticosteroids (ICS) are currently the most effective anti-inflammatory asthma drugs available (1, 10, 11). Fluticasone propionate (FP) is a new ICS with a higher topical anti-inflammatory effect than other ICS. Comparative studies indicate a 2:1 clinical potency ratio of FP compared to beclomethasone dipropionate (BDP) and budesonide, evaluated by BHR, forced expiratory volume in 1 s (FEV₁), peak expiratory flow rates (PEFR), and symptom scores (12, 13). Besides, FP has negligible oral bioavailability, possibly leading to fewer systemic adverse effects (14).

The aim of this study was to determine the state of activation of inflammatory cells in the peripheral blood of adult symptomatic patients with asthma and compare it with that of normal subjects, and to correlate this activation with serum ECP levels, lung-function parameters, and BHR, in the search for a relatively easy, though reliable test with which the clinician may determine the severity of patients' asthma. In addition, we investigated whether these parameters also respond to the anti-inflammatory treatment with inhaled corticosteroids, measuring the influence of inhaled FP (375 µg twice daily) and inhaled BDP (750 µg twice daily) on these parameters. We propose that half the dose of FP affords the same anti-inflammatory effects as BDP.

Material and methods

Design and treatment

The study was a randomized, crossover trial. A schematic overview is shown in Fig. 1. Two 3-week, single-blind, placebo/washout periods were followed by two 6-week, double-blind, active-treatment periods. The two placebo (washout) periods were regarded as the baseline before the active-treatment periods. After the run-in period (the first 3-week placebo period), patients needed, for inclusion, to be symptomatic, defined as having ≥ 4 symptom days during the last week, or ≥ 7 symptom days during the last 2 weeks of the run-in period. Symptom days were defined as days on which any asthma symptom was recorded on a diary card by patients during that day. If so, they randomly received FP 750 µg daily or BDP 1500 µg daily for 6 weeks.

Throughout the study, the patients took three inhalations of the study medication twice daily, administered with a metered-dose inhaler (MDI) containing either placebo, FP, or BDP. The patients used salbutamol MDI (100 µg) p.r.n. as rescue medication. Patients were shown how to use their MDI correctly. No other pulmonary medication was allowed.

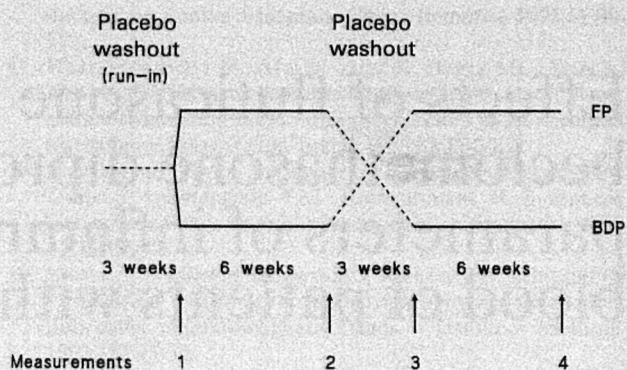


Fig. 1. Design of study. FP: fluticasone propionate 750 µg daily; BDP: beclomethasone dipropionate 1500 µg daily.

Subjects

Thirty adult nonsmoking patients with asthma diagnosed by the criteria of the American Thoracic Society (15) were recruited from the outpatient clinic. Baseline characteristics of these subjects are shown in Table 1. The asthma patients had marked BHR and moderate airways obstruction without their ICS (Table 1). Most patients were allergic, defined as raised specific IgE levels or positive skin tests for house-dust mite or two of seven other common aeroallergens tested (16). All but one used ICS (beclomethasone or budesonide) before entry, with an average daily dose of 790 ± 54 µg (mean $\pm$ SEM). None of the patients had used systemic corticosteroids in the 6 months preceding the study, and no patient had had an upper or lower respiratory tract infection within 6 weeks before the study. For comparison of laboratory data, venous blood was obtained from six normal nonsmoking, nonasthmatic subjects (two men, four women, mean age 28 years). Controls were all nonallergic. The study was approved by the Nijmegen University medical ethics committee. All study subjects gave written informed consent before participating in the study.

Measurements

Laboratory evaluation. At the end of each of the four periods, a venous blood sample was taken, without tourniquet, between 0700 and 0900 for immunophenotyping of inflammatory cells and measurement of total number of eosinophils and ECP levels. These venapunctures always occurred at the same time of the day for the same patients.

The T-cell subsets CD3 (T cells), CD4 (T-helper cells), and CD8 (suppressor/cytotoxic T cells) were measured. Furthermore, the lymphocyte activation markers HLA-DR, CD25 (IL-2 receptor α -chain) and CD49a (very late antigen 1 [VLA-1]), and

Table 1. Baseline characteristics of patients

Subject no.	Sex M/F	Age (years)	Atopic	FEV ₁ * % pred.	FEV ₁ † % pred.	PC ₂₀ H [‡] mg·ml ⁻¹	CD3/HLA-DR [§] % positive	ECP mg·l ⁻¹
1	M	29	Yes	73	72	0.05	8	33
2	M	18	Yes	98	81	0.28	4	112
3	F	28	Yes	95	66	0.27	6	140
4	F	29	Yes	82	73	0.14	4	24.4
5	M	42	Yes	118				
6	M	55	Yes	86	81	3.29	12	2.55
7	M	27	Yes	72	72	0.21	28	6.57
8	M	31	Yes	83				
9	M	25	Yes	62	62	0.05	3	20.4
10	F	34	No	59	59	0.11	17	28.9
11	F	42	Yes	121	112	0.28	4	15.8
12	F	28	No	82	53	0.19	8	3.99
13	F	26	Yes	124	103	0.10	4	17.3
14	F	22	Yes	59	73	0.17	3	64.8
15	F	29	Yes	84	55	0.01	2	57
16	M	28	Yes	51				
17	F	33	Yes	112	91	0.12	3	72
18	M	29	Yes	72				
19	M	40	Yes	79	84	0.24	11	21.6
20	M	23	No	96	88	2.78		9.93
21	M	30	Yes	83	64	0.09		51.6
22	F	29	Yes	62				
23	F	31	No	99				
24	F	29	No	87				
25	M	26	Yes	87	66	0.015	2	34.5
26	F	38	Yes	62	51	0.015	4	45.6
27	F	26	Yes	103	109	0.09	4	79.7
28	M	27	Yes	84	54	0.31	10	32.6
29	F	34	Yes	91				
30	F	20	Yes	72				
Mean		30.3		84.8	74.7 [†]	0.36 [‡]	6.5 [§]	41.6
SD		7.4		18.8	18.1	0.76	6.5	36.7

*At start of study ($n=30$); †after first placebo period (run-in) ($n=21$). Allergy: defined as raised specific IgE levels or positive skin tests for house-dust mite or two of seven other common aeroallergens tested (16); FEV₁: forced expiratory volume in 1 s; PC₂₀H: provocative concentrations of histamine causing 20% fall in FEV₁; ECP: eosinophil cationic protein.

memory (CD45RO) and naive (CD45RA) lymphocyte subtypes were determined in whole blood. Staining was performed by a standard method (17). Briefly, immediately after sampling, 100 µl of heparinized whole blood was incubated at room temperature for 15 min with the appropriate concentration of monoclonal antibodies (mAbs) labeled with phycoerythrin (PE) or fluorescein isothiocyanate (FITC). The following FITC- or PE-conjugated mAbs were used: UCHT1 (CD3), MT310 (CD4), DK25 (CD8), CR3/43 (HLA-DR), UCHL-1 (CD45RO) (Dakopatts, Glostrup, Denmark), Leu 18 (CD45RA) (Becton Dickinson, Mountain View, CA, USA), and ACT-1 (CD25) (Coulter Corporation, Hialeah, FL, USA) and TS2/7 (VLA-1) (T-Cell Sciences Inc., Cambridge, MA, USA). Erythrocytes were subsequently lysed in FACS lysing reagent (Becton Dickinson, Mountain View, CA, USA) for 10 min at room temperature. After lysis, the cells were washed in phosphate-buffered saline

(PBS) and fixed in 1% paraformaldehyde solution. Cells were analyzed with a Coulter XL flow cytometer (Coulter Corporation, Hialeah, FL, USA). Results were expressed as percentage of cells within the corresponding lymphocyte cluster, which was corrected for monocytes. For comparison, six normal nonasthmatic subjects were also measured according to the same protocol.

Total numbers of eosinophils were measured with a Technicon H₁ analyzer (Technicon Instrument Co., Tarrytown, NY, USA). Blood samples for ECP were left for exactly 1 h to clot at room temperature; after centrifugation, the serum was stored at -70°C to be analyzed with the Pharmacia CAP System[®].

Lung function and BHR. At the end of each of the four periods, lung-function measurements and the histamine provocation test were performed. Subjects did not use rescue and study medication at least 8 h before each visit and rested for 15 min

before the start of the measurements. Lung function and responses to provocation with histamine ($PC_{20}H$) were assessed with a flow-volume curve recorded on a heated pneumotachograph (Spiro analyzer ST 250®; Fukuda Sangyo Co., Tokyo, Japan). Baseline lung function was recorded as the best of three reproducible values of FEV_1 (within 5%) before the histamine provocation test. The histamine provocation test, carried out by the method of Cockcroft et al. (18), was reported previously (13).

Statistical analysis

In this study, four repeated measurements were made, at the start and at the end of each of both treatment periods (measurements 1 to 4, ordered in time) (13). The carry-over effect of the first treatment at the start of the second treatment period (measurement 3) is a first-order carry-over effect, which may still be partly present at the end of the second treatment period (measurement 4). This latter part is the second-order carry-over effect, which may interfere with the treatment effect. The first-order carry-over effect was tested by comparing the differences of measurements 3 and 1 between the two randomized treatment groups (*t*-test for independent samples or Mann-Whitney U-test whenever appropriate). Theoretically, the second-order carry-over effect is tested by comparing the sum of measurements 2 and 4 between the treatment-order groups (19). However, in the present study, by misfortune (dropouts) randomization groups were not equal, leading to a significant difference in "grand mean" μ (Levine's test for equality of variances) in some outcome variables (e.g., absolute number of eosinophils) between the two treatment groups at the start of the study. As the dropouts occurred by prerandomization criteria, this did not interfere with the validity of the study. To correct for this imbalance present at measurement 1, all tests should be made independent of the "grand mean". Now, the second-order carry-over effect was tested by comparing the sum of two differences (i.e., 2 minus 1 and 4 minus 1) between the two treatment-order groups (19).

Provided that there was no second-order carry-over effect, the treatment effect between FP and BDP was tested next by comparing the difference of measurements 2 and 4 between the treatment-order groups. The treatment effect "FP minus BDP" was estimated by taking half the difference between the two treatment-order groups of the within-group mean differences of measurements 2 and 4: $([FP-BDP]) - ([BDP-FP])/2$ (13, 19). The treatment effect of both ICS (Δ -effect) was also expressed by taking the differences between real

baseline values and the corresponding values after treatment.

FEV_1 data are shown as percentage of predicted. The $PC_{20}H$ data were $\log_2$ -transformed before analysis and expressed as doubling doses (DD) of inhaled histamine. The Wilcoxon signed rank test or the paired *t*-test was used for analysis if appropriate. The Mann-Whitney U-test was used to compare data between groups. Correlations were calculated by means of the Spearman correlation test. *P* values less than 0.05 were considered significant. Data are reported as mean values $\pm$ SEM or median (range).

Results

Thirty patients started with the first placebo (run-in) period. Two patients did not meet the final criteria after the run-in period, because they experienced no asthma symptoms during that period. Seven patients dropped out in the run-in period because of exacerbation of their asthma after withdrawal of their ICS. The remaining 21 patients all completed the study. Of these patients, 14 started with BDP and seven with FP.

There were no first- or second-order carry-over effects for any variable measured. None of the parameters were significantly different between the two baseline periods.

Laboratory evaluation

The percentage of CD3/HLA-DR+ cells was significantly increased in the peripheral blood of asthmatics compared to normal subjects, after both placebo and therapy with ICS (Table 2). The percentage of CD3/HLA-DR+ cells decreased from 10.6 ± 2.06 to 5.43 ± 0.86 after treatment with FP ($P < 0.05$), and from 8.11 ± 1.59 to 4.81 ± 0.85 after BDP (NS) (Fig. 2). No increase in other lymphocyte activation markers was found in comparison with normal subjects. Neither FP nor BDP influenced these markers (Table 2). Direct treatment comparison (see statistical section) showed no differences between the effects of FP and BDP on any marker.

ECP decreased from 36.7 ± 6.28 to $19.9 \pm 3.57 \mu g \cdot l^{-1}$ ($P < 0.001$) after BDP and from 34.2 ± 7.45 to 23.4 ± 3.90 (NS) after FP (Table 3, Fig. 2). Direct treatment comparison did not show significant differences between FP and BDP (difference in treatment effect FP-BDP of 3.21 ± 2.3 ; 95% confidence interval ([CI] -1.68 to 8.10 [NS])).

The total numbers of eosinophils in the peripheral blood decreased from 0.45 to $0.22 \times 10^9 \cdot l^{-1}$ ($P < 0.001$) after BDP, and from 0.41 to $0.30 \times 10^9 \cdot l^{-1}$ ($P < 0.01$) after FP (Table 3, Fig. 2). The decrease in total numbers of eosinophils was significantly

Table 2. Immunophenotyping of T cells

Cells (% positive)	Normal subjects	ICS	Baseline median (range)	6-week therapy median (range)	P value [†]
CD3	77.0 (25.0)	FP	73.0 (58.0)	73.0 (36.0)	NS
		BDP	69.5 (26.0)	71.0 (61.0)	NS
CD4	41.5 (10.0)	FP	46.0 (38.0)	46.0 (25.0)	NS
		BDP	46.0 (44.0)	45.0 (27.0)	NS
CD8	34.5 (12.0)	FP	32.0 (36.0)	30.0 (33.0)	NS
		BDP	30.5 (32.0)	31.0 (37.0)	NS
CD3/HLA-DR	0.5 (6.0)	FP	8.5 (38.0)**	5.0 (16.0)*	0.03
		BDP	4.0 (27.0)**	4.0 (15.0)*	NS
CD4/CD25	4.0 (6.0)	FP	8.0 (17.0)	6.0 (11.0)	NS
		BDP	5.02 (10.0)	4.0 (15.0)	NS
CD8/CD25	0.0 (2.0)	FP	0.0 (4.0)	0.0 (4.0)	NS
		BDP	0.0 (7.0)	0.0 (8.0)	NS
CD4/VLA1	0.0 (2.0)	FP	1.0 (3.0)	1.0 (4.0)	NS
		BDP	0.0 (3.0)	0.0 (2.0)	NS
CD8/VLA1	0.0 (1.0)	FP	1.0 (3.0)	0.0 (7.0)	NS
		BDP	0.0 (4.0)	0.0 (8.0)	NS
CD4/CD45RA	17.5 (12.0)	FP	22.0 (34.0)	19.0 (34.0)	NS
		BDP	21.0 (28.0)	20.0 (12.0)	NS
CD4/CD45RO	20.0 (12.0)	FP	23.0 (26.0)	23.0 (21.0)	NS
		BDP	23.0 (50.0)	25.0 (21.0)	NS

[†] P values of Δ -treatment effects were calculated as values after 6 weeks of treatment vs corresponding baseline values (Wilcoxon signed rank test). NS: not significant. Comparison of values with those of six normal subjects was performed by Mann-Whitney U-test: * $P < 0.05$; ** $P < 0.01$.

different between FP and BDP (mean difference in treatment effect FP-BDP 0.076 ± 0.07 ; 95% CI 0.024 to 0.13; $P < 0.01$).

BHR and lung function

Both FP and BDP caused a significant decrease in BHR and an increase in lung function (Table 3). There were no significant differences between the effects of FP and BDP on BHR and lung function.

Correlations

The change in percentage of CD3/HLA-DR+ positive cells after treatment with BDP correlated with the change in ECP ($r = 0.46$; $P < 0.05$), but not after treatment with FP. At all measurement points, ECP correlated with the number of eosinophils in the peripheral blood (after both placebo periods, $r > 0.72$, $P < 0.001$; after treatment with FP, $r = 0.70$, $P < 0.001$; and after treatment with BDP, $r = 0.59$, $P = 0.005$), but not with $PC_{20}H$ and FEV_1 , nor did the change in ECP correlate with changes in clinical parameters. Neither analyzing ECP values above the normal value of $20 \mu\text{g} \cdot \text{l}^{-1}$ (20), nor analyzing parallel ECP values (only measurements 1 and 2 in time), improved correlation coefficients.

Discussion

The aim of this study was to determine the state of activation of inflammatory cells in the peripheral

blood of adult symptomatic patients with asthma, and the effects of ICS on these parameters. Only activated (CD3/HLA-DR+) lymphocytes were increased in percentage in the peripheral blood, compared to normal subjects. The percentage of CD3/HLA-DR+ lymphocytes decreased by almost 50% after treatment with clinically efficacious doses of ICS. ECP levels were increased in patients without ICS (Fig. 2), decreasing after treatment to the upper limit of the normal level ($20 \mu\text{g} \cdot \text{l}^{-1}$). There were no clear correlations between inflammatory parameters, ECP, and BHR and lung function. No significant differences in effects were found between FP 750 μg daily and BDP 1500 μg daily.

The basic hallmark of asthma is chronic inflammation in the airways (1–3), characterized by activated lymphocytes and eosinophils, which persists even when patients have few or no symptoms (21, 22). Invasive procedures such as bronchial biopsies and bronchoalveolar lavage (BAL) studies are required to measure inflammatory cells and markers directly, and these markers do correlate with disease severity and clinical symptoms (21–23). To assess this inflammation more easily, only indirect measurements such as lung function or inhalation challenges have been performed so far. However, signs of this inflammation can also be detected in the peripheral blood of asthmatic subjects (6–8), and serum ECP has been promoted as a direct marker of this eosinophilic inflammation (9).

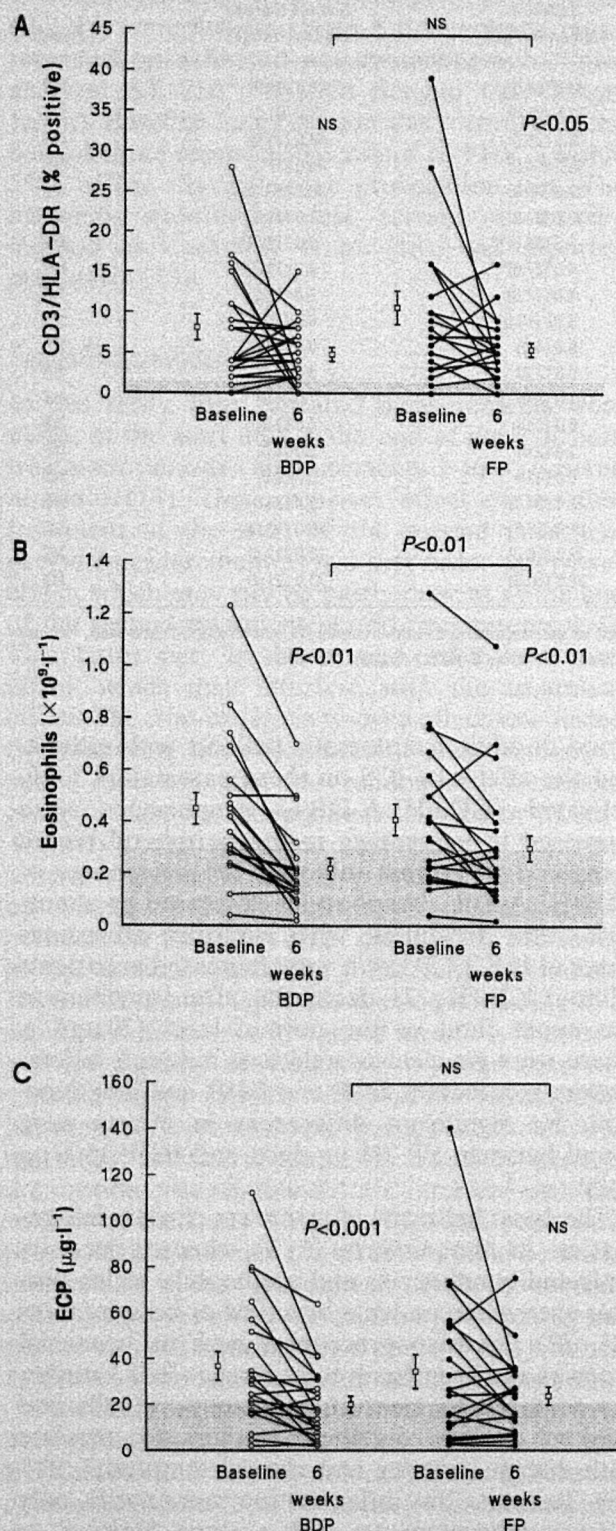


Fig. 2. Changes in individual HLA-DR+ cells (A), absolute numbers of eosinophils in peripheral blood (B), and eosinophil cationic protein (ECP) (C) values from baseline to 6 weeks after treatment and mean $\pm$ SEM ($\square$) with fluticasone propionate (FP) 750 $\mu\text{g/day}$ ($\bullet$) and beclomethasone dipropionate (BDP) 1500 $\mu\text{g/day}$ ($\circ$). Lower limit of normal is indicated at bottom of figure. NS: not significant.

Table 3. Clinical efficacy of FP and BDP

Parameter	Normal values	ICS	Baseline mean (SEM)	6-week therapy mean (SEM)	P value [†]
ECP	<20 mg l^{-1}	FP	34.2 $\pm$ 7.45	23.4 $\pm$ 3.90	NS
		BDP	36.7 $\pm$ 6.28	19.9 $\pm$ 3.57	***
Eosinophils	<0.30 $\times 10^9 \text{ l}^{-1}$	FP	0.41 $\pm$ 0.06	0.30 $\pm$ 0.05	**
		BDP	0.45 $\pm$ 0.06	0.22 $\pm$ 0.04	**
PC ₂₀ H	<8 mg ml^{-1}	FP	0.41 $\pm$ 0.17	2.42 $\pm$ 0.17	***
		BDP	0.49 $\pm$ 0.24	1.63 $\pm$ 0.61	***
FEV ₁	100% predicted	FP	74.1 $\pm$ 4.25	86.9 $\pm$ 3.33	**
		BDP	76.4 $\pm$ 3.51	85.8 $\pm$ 3.14	**

FP: fluticasone propionate; BDP: beclomethasone dipropionate. For other abbreviations, see Table 1. [†] Δ -treatment effects were calculated as treatment values vs corresponding baseline values (Wilcoxon signed rank test). NS: not significant; ** $P < 0.01$; *** $P < 0.001$.

T cells play an important role in the orchestration of the inflammation in the lung of allergic asthmatics (3, 24). In our study, lymphocyte subsets in the peripheral blood in adult symptomatic patients with moderate to severe BHR were like those of normal subjects (Table 2). Only the percentage of CD3/HLA-DR positive lymphocytes was increased compared to normal subjects. Other increases in lymphocyte activation markers were not found. Absolute numbers of cells of each phenotype might be more revealing in terms of therapy-induced changes, but, as this was a cross-over trial, with a within-patient comparison, and the numbers of lymphocytes remained stable at the different time points within patients, absolute numbers showed exactly the same results, and data were not shown. The same was true for the surface density of receptors measured as the median of the absolute fluorescence, which also showed only an increase in expression of CD3/HLA-DR. In a previous study, CD4-positive T cells in the peripheral blood of patients with acute exacerbation of their asthma demonstrated an increased expression of the activation markers HLA-DR, CD25, and the integrin CD49a (VLA-1), compared to normal subjects (6). These increases diminished with therapy and improvement in lung function (6). However, these patients were admitted to the hospital because of an acute exacerbation with presumably large upregulation of their inflammation. Activation of peripheral blood T cells also occurred in patients with milder asthma, although levels in BAL were much higher (7, 8). In patients similar to ours, Wilson et al. (23) showed a marked upregulation of expression of activation markers such as CD25 and HLA-DR in BAL fluid, but only upregulation of HLA-DR expression in the peripheral blood, a condition which responded to treatment with 2000 μg of BDP daily for 2 weeks (23). This is in line with the findings in the present study, with

an increase only in CD3/HLA-DR+lymphocytes. The extent of inflammation in the peripheral blood appears at most a small reflection of the acute inflammation within the lung, which can be assessed in BAL fluid and biopsies. Furthermore, the washout period from ICS is probably not long enough. Besides, most of our patients were atopic, and atopic patients appear to have significantly lower levels of activation markers than nonatopic patients (6, 7). Still, the percentage of HLA-DR+lymphocytes was found to be increased in the peripheral blood, responding significantly to treatment with FP 750 µg daily. The number of CD3/HLA-DR+ lymphocytes also decreased by almost 50% after therapy with BDP (1500 µg daily), but this was not significant. This may be due to the great standard deviation of the measured parameter. Direct treatment comparison showed no differences between the effects of FP and BDP. Despite treatment with ICS, CD3/HLA-DR+lymphocytes remained significantly elevated compared to the normal subjects.

No increase was seen in CD25 or VLA-1 on T-helper or T-suppressor cells in the peripheral blood, with no decrease after treatment with both ICS. The memory phenotype (CD45RO+) of lymphocytes was found to be increased in BAL fluid and biopsies (8, 25), whereas both CD45RO and CD45RA were not changed in peripheral blood (8). This is consistent with the present study.

As in the study by Wilson et al. (23), we did not find significant correlations between inflammatory markers, BHR, and lung function in the peripheral blood, correlations that could be found in BAL fluid (8).

ECP can be directly quantitated in serum and this possibly relates to priming of eosinophils. Elevated serum ECP levels may reflect airway inflammation, and they are possibly related to the severity of asthma (9). ECP was raised in our patients after treatment with placebo for 3 weeks, and decreased after treatment with both ICS to the upper value of normal ($20 \mu\text{g}\cdot\text{l}^{-1}$). In the treatment comparison of FP and BDP, they were directly compared by analyzing the difference of the treatment effects of FP and BDP (between the treatment-order groups $([\text{FP}-\text{BDP}] - ([\text{BDP}-\text{FP}])/2$). BDP offered a statistically significant Δ -treatment effect, in contrast to FP. This may be because only seven patients started on FP, when ECP levels were higher than after the second placebo period (14 patients). Still, because FP also decreased ECP levels by half, the treatment comparison of FP and BDP was not statistically significantly different between both drugs. In contrast to previous reports (9, 20), ECP did not correlate with clinical para-

meters and BHR, nor did the change in ECP correlate with changes in clinical parameters, and therefore did not reflect the severity of asthma in the present study. Ferguson et al. (26) also concluded that serum ECP was a poor test to identify persistent inflammation or to modify therapy in children with chronic asthma, in contrast to BHR or lung function.

In our study, ECP levels did correlate very well with the absolute eosinophil count. Primed eosinophils release ECP upon clotting. The quantity that is released depends on the physical circumstances in which serum collection takes place, such as ambient temperature, time allowed for clotting, centrifugation, and diurnal variation (26). Therefore, we carefully standardized the measurements of ECP, according to the recommendations of the supplier of the ECP kit. Similarly, the quantity of ECP released may also depend on the extent of activation of peripheral blood eosinophils in response to eosinophilic inflammation in the lungs (27), and serum ECP may be a good marker for primed eosinophils. Eosinophil suppression in peripheral blood, as found in our study, may well occur through reduced cytokine production as IL-5 by activated T cells. IL-5 has clearly been implicated in the pathogenesis of asthma, by its promotion of the differentiation, priming, and survival of eosinophils *in vitro* (2). In a proportion of patients with chronic severe asthma, IL-5 is detectable in serum (28), although this may not reflect the presence of IL-5 within the bronchial mucosa. Furthermore, IL-5 decreases after treatment with ICS, in association with a significant fall in peripheral blood eosinophil counts (29). This may be because of the inhibition of release of cytokines by ICS locally within the airways with less "spillover" into the circulation, by recirculation of unactivated T cells from the airways into the circulation, or by a direct effect of ICS on activated T cells in the peripheral blood (23, 29, 30). On the other hand, the decrease in ECP levels may be due to direct (systemic) effects of high doses of ICS on eosinophils, leading to a decrease in eosinophils in the peripheral blood by recruitment of the eosinophils from the circulation, decrease in survival time, and suppression of bone marrow (30). Blood eosinophils are very sensitive to the systemic effects of steroids (31). FP has a very low oral bioavailability and great first-pass metabolism (12, 14). In this way, FP showed less systemic activity than BDP. Absolute numbers of eosinophils decreased significantly less after FP than after BDP ($P < 0.01$). This may also explain the smaller decrease in ECP values after treatment with FP. Still, serum ECP was increased in our symptomatic asthma patients, and it decreased to (near) normal values after treatment.

In conclusion, activated T cells can be found in the peripheral blood of symptomatic patients with asthma, and they are reduced by inhaled corticosteroids at clinically efficacious doses. FP 750 µg daily is at least as effective as BPD 1500 µg daily in reducing HLA-DR+ lymphocytes and BHR, and increasing FEV₁. Serum ECP has been found to be increased in symptomatic patients with asthma, and it may be a good marker of primed eosinophils. Serum ECP values decrease after treatment with both ICS. No clear correlations were found between inflammatory parameters, ECP, BHR, and lung function.

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References

1. National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland. International consensus report on diagnosis and treatment of asthma. *Eur Respir J* 1992;5:601-41.
2. Kay AB. Asthma and inflammation. *J Allergy Clin Immunol* 1991;87:893-910.
3. Rochester CL, Rankin JA. Is asthma T-cell mediated? *Am Rev Respir Dis* 1991;144:1005-7.
4. Robinson DS, Hamid Q, Ying S, et al. Predominant T_{H2}-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298-304.
5. Walker C, Virchow JC Jr, Bruijnzeel PLB, Blaser K. T cell subsets and their soluble products regulate eosinophilia in allergic and nonallergic asthma. *J Immunol* 1991;146:1829-35.
6. Corrigan CJ, Kay AB. CD4 T-lymphocyte activation in acute severe asthma. Relationship to disease severity and atopic status. *Am Rev Respir Dis* 1990;141:970-7.
7. Walker C, Bode E, Boer L, Hansel TT, Blaser K, Virchow JC Jr. Allergic and nonallergic asthmatics have distinct patterns of T-cell activation and cytokine production in peripheral blood and bronchoalveolar lavage. *Am Rev Respir Dis* 1992;146:109-15.
8. Robinson DS, Bentley AM, Hartnell A, Kay AB, Durham SR. Activated memory T helper cells in bronchoalveolar lavage fluid from patients with atopic asthma: relation to asthma symptoms, lung function, and bronchial hyperresponsiveness. *Thorax* 1993;48:26-32.
9. Venge P. Serum measurements of eosinophilic cationic protein (ECP) in bronchial asthma. *Clin Exp Allergy* 1993; 23 Suppl 2:3-7.
10. Guidelines on the management of asthma. *Thorax* 1993;48 Suppl:S1-24.
11. Barnes PJ, Pedersen S. Efficacy and safety of inhaled corticosteroids in asthma. *Am Rev Respir Dis* 1993;148 Suppl:S1-26.
12. Holliday SM, Faulds D, Sorkin E. Inhaled fluticasone propionate. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in asthma. *Drugs* 1994;47:318-31.
13. Bootsma GP, Dekhuijzen PNR, Festen J, Mulder PGH, van Herwaarden CLA. Comparison of fluticasone propionate and beclomethasone dipropionate on direct and indirect measurements of bronchial hyperresponsiveness in patients with stable asthma. *Thorax* 1995;50: 1044-50.
14. Bootsma GP, Dekhuijzen PNR, Festen J, Mulder PGH, Swinkels LMJW, van Herwaarden CLA. Fluticasone propionate does not influence bone metabolism in contrast to beclomethasone dipropionate. *Am J Respir Crit Care Med* 1996;153:924-30.
15. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. *Am Rev Respir Dis* 1987; 136:225-44.
16. Oosterhoff Y, Koëter GH, de Monchy JGR, Postma DS. Circadian variation in airway responsiveness to methacholine, propranolol, and AMP in atopic asthmatic subjects. *Am Rev Respir Dis* 1993;147:512-17.
17. Reichert T, DeBruyère M, Deneys V, et al. Lymphocyte subset reference ranges in adult Caucasians. *Clin Immunol Immunopathol* 1991;60:190-208.
18. Cockcroft DW, Killian DN, Mellon JA, Hargreave FE. Bronchial reactivity to inhaled histamine; a method and clinical survey. *Clin Allergy* 1977;7:235-43.
19. Jones B, Kenward MG. Design and analysis of cross-over trials. London: Chapman and Hall, 1989:16-88.
20. Wever AMJ, Wever-Hess J, Hensgens HESJ, Hermans J. Serum eosinophil cationic protein (ECP) in chronic asthma. Relationship to spirometry, flow-volume curves, PC₂₀, and exacerbations. *Respir Med* 1994;88:613-21.
21. Jeffery PK, Wardlaw A, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma: an ultrastructural quantification study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989;140:1745-53.
22. Azzawi M, Bradley B, Jeffery PK, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthma. *Am Rev Respir Dis* 1990;142:1407-13.
23. Wilson JW, Djukanovic R, Howarth PH, Holgate ST. Inhaled beclomethasone dipropionate downregulates airway lymphocyte activation in atopic asthma. *Am J Respir Crit Care Med* 1994;149:86-90.
24. Kay AB. "Helper" (CD4⁺) T cells and eosinophils in allergy and asthma. *Am Rev Respir Dis* 1992;145 Suppl: S22-6.
25. Sont JK, van Krieken JM, Evertse CE, Hooijer R, Willems LNA, Sterk PJ. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity in patients treated with inhaled steroids. *Thorax* 1996; 51:496-502.
26. Ferguson AC, Vaughan R, Brown H, Curtis C. Evaluation of serum eosinophilic cationic protein as a marker of disease activity in chronic asthma. *J Allergy Clin Immunol* 1995;95:23-8.
27. Ahlstedt S, Enander I, Petersen CGB, Lanner A. The clinical assessment of the inflammatory component in asthma with emphasis on the eosinophils. *Pract Allergy Immunol* 1993;8:149-54.
28. Alexander AG, Barkans J, Moqbel R, Barnes NC, Kay AB, Corrigan CJ. Serum interleukin 5 concentrations in atopic and non-atopic patients with glucocorticoid-dependent chronic severe asthma. *Thorax* 1994;49:1231-3.
29. Corrigan CJ, Haczku A, Gemou-Engesaeth V, et al. CD4 T-lymphocyte activation in asthma is accompanied by increased serum concentrations of interleukin-5. Effect of glucocorticoid therapy. *Am Rev Respir Dis* 1993; 147:540-7.

30. Evans PM, O'Conner BJ, Fuller RW, Barnes PJ, Chung KF. Effect of inhaled corticosteroids on peripheral blood eosinophil counts and density profiles in asthma. *J Allergy Clin Immunol* 1993;91:643-50.
31. Toogood JH, Baskerville JC, Jennings B, Lefcoe NM, Johansson SA. Influence of dosing frequency and schedule on the response of chronic asthmatics to the aerosol steroid budesonide. *J Allergy Clin Immunol* 1982;70:288-98.

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