

Control of airway inflammation maintained at a lower steroid dose with 100/50 µg of fluticasone propionate/salmeterol

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Background: Inhaled corticosteroids (ICSs) have been shown to reverse epithelial damage and decrease lamina reticularis thickness in patients with asthma.

Objective: This study investigated whether clinical asthma control and airway inflammation could be maintained after switching therapy from medium-dose fluticasone propionate (FP) to low-dose FP administered with the long-acting β_2 -agonist (LABA) salmeterol.

Methods: Eighty-eight subjects (age, ≥ 18 years) who, during open-label screening, demonstrated improved asthma control after an increase from 100 µg of FP twice daily to 250 µg of FP twice daily were randomized to receive 100/50 µg of FP/salmeterol through a Diskus inhaler (GlaxoSmithKline, Research Triangle Park, NC) twice daily or continue 250 µg of FP twice daily through a Diskus inhaler for 24 weeks. Clinical outcomes were monitored, and bronchial biopsy specimens and bronchoalveolar lavage fluid were obtained before and after 24 weeks of treatment.

Results: There were no significant differences between treatments with respect to eosinophils in the bronchial mucosa and bronchoalveolar lavage fluid; mucosal mast cells, neutrophils, or CD3⁺, CD4⁺, CD8⁺, or CD25⁺ T lymphocytes; or concentration of mediators (GM-CSF, IL-8, and eosinophil cationic protein). The 2 treatments were not different with respect to lamina reticularis thickness. Consistent with the airway inflammatory measures, clinical and physiologic measures of asthma control were also similar.

Conclusion: This study demonstrates that control of asthma and airway inflammation is maintained over the 24-week treatment period when patients requiring a medium-dose ICS are switched to a lower-dose ICS with a LABA.

Clinical implications: A lower-dose ICS with a LABA is effective in controlling inflammation and providing clinical asthma control, confirming current guideline recommendations. (J Allergy Clin Immunol 2006;118:44-52.)

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Asthma is a chronic airway disorder characterized by inflammation and remodeling that results in airway hyperresponsiveness and variable airflow limitation. Inhaled corticosteroids (ICSs), the mainstay of asthma management, modulate numerous cells and mediators known to be involved in the pathogenesis of asthma, reducing the numbers of airway eosinophils, T cells, and macrophages and mRNA expression for proinflammatory mediators (eg, IL-4 and IL-5) and adhesion molecules (eg, intercellular adhesion molecule 1 and E-selectin).^{1,2} Furthermore, ICSs have been shown to reverse epithelial damage and decrease lamina reticularis thickness in the airways of patients with asthma.³⁻⁵ Although the diverse anti-inflammatory effects of ICSs in the treatment of asthma are well established, their long-term use can be associated with systemic side effects, particularly when they are used at high doses for prolonged periods of time. Thus studies have shown a dose relationship between ICSs and a reduction in bone density.⁶ This, together with the possibility of other effects on the skin and eyes,⁷ has led to the recommendation that the dose of ICS should be reviewed regularly and reduced to the minimum that controls disease effectively.

It is recognized that strategies to reduce the ICS dose must also effectively control the underlying airway inflammation that typifies asthma. Studies have shown that the addition of a long-acting β_2 -agonist (LABA) to a low dose of ICS does not lead to clinical improvement at the expense of worsening inflammation, as demonstrated by the equivalent anti-inflammatory potency of high-dose ICSs alone and combination therapy.³ However, there have been no studies to date comparing control of airway inflammation in patients treated with a moderate dose of an ICS with that in patients receiving a combination of a low-dose ICS and a LABA, a therapeutic option that is increasingly considered in an attempt to reduce the risk of ICS-associated side effects. Excessive reductions in the dose of an ICS in the presence of a LABA lead to a swift worsening of inflammation that can precede clinical signs of worsening asthma.⁸⁻¹⁰

To assess the safety and efficacy of switching patients from a moderate-dose ICS to a low-dose ICS combined with a LABA, we have conducted the largest bronchoscopy study in asthma reported to date. Bronchial biopsy and bronchoalveolar lavage (BAL) were performed before and after the switch to monitor the effects on submucosal eosinophils, the primary outcome variable, and a series of other cell and mediator indices selected for their reported roles in asthma.

METHODS

Prospectively, 88 subjects underwent endobronchial biopsy and BAL at selected sites as part of this larger replicate study that was similar in overall design to the study previously reported by Busse et al.¹¹ Briefly, this was a randomized, double-blind, parallel-group study that evaluated patients with asthma who, during the first part of the run-in period (run-in period 1), had stable symptoms with their

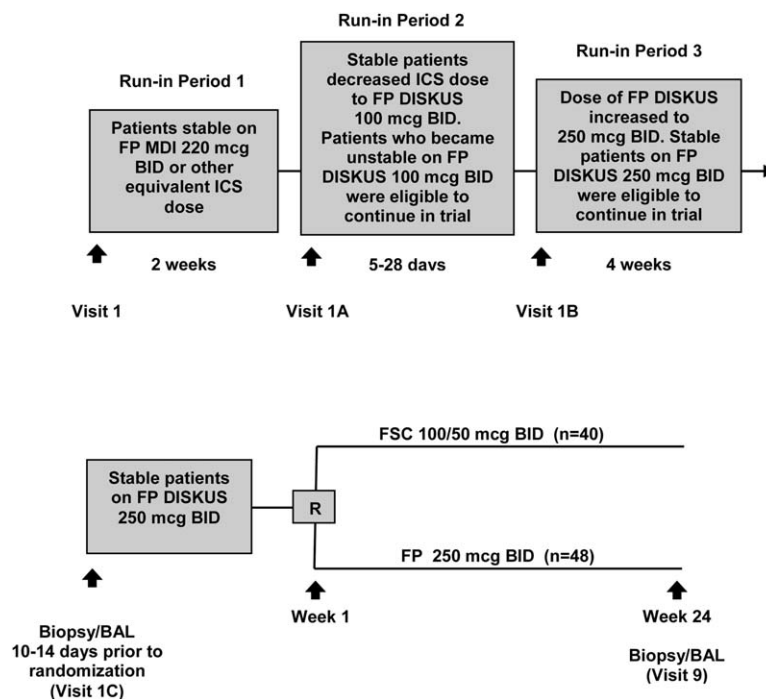
Abbreviations used

BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
COPD:	Chronic obstructive pulmonary disease
ECP:	Eosinophil cationic protein
FP:	Fluticasone propionate
FSC:	Fluticasone propionate/salmeterol
ICS:	Inhaled corticosteroid
IQR:	Interquartile range
LABA:	Long-acting β_2 -agonist
PEF:	Peak expiratory flow

prestudy medium doses of ICS (220 μ g of fluticasone propionate [FP] twice daily or equivalent) but whose asthma destabilized during a subsequent run-in period after a dose step-down to 100 μ g of FP twice daily (run-in period 2). During the final open-label run-in period, treatment was stepped up to 250 μ g of FP twice daily for 4 weeks (run-in period 3), at which time all patients had to re-establish asthma control to be included in the treatment phase of the study (Fig 1). This meant that subjects were not allowed to continue if they met any of the following asthma instability criteria that were also stated by Busse et al¹¹: a 20% or greater decrease from the screening visit predose FEV₁; a 20% or greater decrease from the mean morning baseline peak expiratory flow (PEF) on any one of 7 days immediately preceding a visit; a total symptom score of 8 or greater during any one week before run-in visit 1B; 18 or more puffs of albuterol during any 1 week before run-in visit 1B; or 2 or more nighttime awakenings caused by asthma requiring treatment with albuterol during any 1-week period. The inclusion criteria included a best FEV₁ within $\pm$ 15% of the best predose screening FEV₁, no more than one nighttime awakening requiring albuterol, and use of 18 or fewer inhalations of albuterol during the previous week. Subjects whose symptoms became stable on 250 μ g of FP twice daily were then randomized to receive either 100/50 μ g of fluticasone propionate/salmeterol (FSC) twice daily or 250 μ g of FP twice daily for 24 weeks.

Subjects were not eligible for study participation if there was current evidence of chronic bronchitis, emphysema, or respiratory diseases other than asthma. In addition, subjects with current tobacco use or a smoking history of more than 10 pack-years were excluded to ensure that those patients with possible chronic obstructive pulmonary disease (COPD) were not enrolled in the study.

All subjects were 18 years of age or older, had a diagnosis of persistent asthma for at least 6 months,¹² had an FEV₁ of 65% to 95% of predicted value (race adjusted for African American subjects), and had at least 30 days of treatment with a medium-dose ICS (504-840 μ g/d beclomethasone dipropionate chlorofluorocarbon; 240-480 μ g/d beclomethasone dipropionate hydrofluoroalkane; 400-800 μ g/d budesonide; 440-660 μ g/d FP [metered-dose inhaler]; 1000-1500 μ g/d flunisolide; or 1200-1600 μ g/d triamcinolone acetonide) or equivalent doses of approved ICSs in Canada. Subjects who experienced an exacerbation after bronchoscopy at run-in visit 1C (n = 5) were rescheduled for the randomization visit 30 days after successful treatment with oral corticosteroids. Any subject who experienced 2 or fewer exacerbations during the study and who was successfully treated with oral corticosteroids continued on the study drug and underwent endobronchial biopsy and BAL at week 24. Subjects who experienced an exacerbation that was not successfully treated with corticosteroids (n = 1) or who experienced 2 or more exacerbations (n = 1) during the treatment period were withdrawn from the study.

FIG 1. Study design. *BID*, Twice daily.

Bronchoscopy and bronchial biopsy procedures

Fiberoptic bronchoscopy was performed at the end of the open-label 250- μ g FP twice daily run-in period (run-in period 3) before starting randomized treatment and after 24 weeks of treatment with either 250 μ g of FP twice daily or 100/50 μ g of FSC twice daily. During bronchoscopy, endobronchial biopsy specimens were obtained from the right middle and lower lobe or lingula and left lower lobe. BAL was then performed with 2 aliquots of 50 mL of 0.9% NaCl solution in the contralateral upper lobe. At least 5 endobronchial biopsy specimens were obtained during each bronchoscopy. The biopsy specimens were taken from the subcarinae, starting with the subsegmental carinae, with subsequent samples being obtained by moving proximally to progressively larger subcarinae.

Bronchial biopsy processing and analyses

Bronchial biopsy specimens were fixed in acetone plus protease inhibitors at -20°C overnight and processed into glycol methacrylate resin the next day, as previously described.¹³ Initially, a survey section was cut and stained by using the rapid toluidine blue method to assess suitability for further analysis. Biopsy specimens that were accepted as adequate for analysis had to have a minimum of 0.46 mm² of lamina propria, excluding smooth muscle, glands, and crushed artifact.¹⁴ Two-micrometer-thick sections were then cut from suitable biopsy specimens and stained immunohistochemically with the streptavidin biotin-peroxidase detection system and mAbs specific for eosinophil cationic protein (ECP; EG2, Pharmacia & Upjohn Diagnostics, Milton Keynes, United Kingdom), tryptase present in mast cells (AA1, DakoCytomation, Ely, United Kingdom), neutrophil elastase present in neutrophils (NP57, DakoCytomation), total T cells (CD3⁺, UCHT1, DakoCytomation), helper T cells (CD4⁺, Lu3a3b, Becton Dickinson, Cowley, United Kingdom), suppressor/cytotoxic T cells (CD8⁺, DK25, DakoCytomation), and activated T cells bearing the IL-2 receptor (CD25⁺, ACT-1,

DakoCytomation). Positively stained nucleated cells were counted in the lamina propria and epithelium. The area of the lamina propria and length of the epithelium were measured by using computer-assisted image analysis (KS400 software; Image Associates, Bicester, United Kingdom), and the number of cells per square millimeter of lamina propria and per millimeter length of intact, longitudinally oriented epithelium were calculated.

In addition, the thickness of the lamina reticularis was measured under intact, longitudinally orientated epithelium. In brief, an image of the lamina reticularis was captured from the toluidine blue-stained survey section and delineated. An Euclidean distance transformation was applied; this identified the central line within the lamina reticularis. A gray level coding was then assigned to each pixel between the central line and the outer boundary, relative to the distance (ie, the higher the gray level, the greater the distance [thickness]). An appropriate scaling for the magnification used was then applied, and the distance in micrometers was calculated.

Bronchoalveolar analyses

BAL fluid (BALF) was obtained as previously described, and IL-8, GM-CSF, and ECP levels were measured by using concentrated BALF with back calculation for the degree of ultrafiltration to "neat" concentrations. BALF was concentrated 1- to 26-fold ($m = 11.24 \pm 0.46$) by means of ultrafiltration through a 5-kd cutoff filter (Amicon Ultra-15 Centrifugal Filter; Millipore Corp, Billerica, Mass), and aliquots were stored at -80°C for measurement of IL-8, GM-CSF, and ECP levels. IL-8 and GM-CSF levels were measured with commercial ELISA kits from R&D Systems (Minneapolis, Minn). ECP levels were measured by using the UniCAP system (Pharmacia Diagnostics). Results are reported per milliliter of unconcentrated BALF.

Statistical analyses

Previous studies indicated that 80 to 85 subjects were needed to discern differences in submucosal eosinophils.^{15,16} The proposed

sample size for this study provided 80% or greater power to ensure that a 95% CI for the difference in eosinophils between treatments was contained within the margin of equivalence (25%), assuming that a 50% increase in eosinophils was considered clinically relevant; 2 one-sided *t* tests for equivalence were used, with an SD of 35%. All statistical analyses were conducted on the population of subjects who had 1 or more successful bronchoscopies. All data from subjects who were withdrawn early were included in the analyses, and no interpolation was done for missing data.

For clinical outcomes, baseline values were calculated as the mean of measurements from the 7 days preceding randomization, and end points were calculated as the mean of measurements from the last week of treatment. The 95% CIs for mean difference in change from baseline at end point for each assessment were obtained from analysis of covariance models that included terms for baseline, treatment, and investigator.

Baseline and end point values for the bronchial biopsy and BAL analyses were derived from the bronchoscopies performed before randomization and at the week 24 visit, respectively. Because of the nonnormality of biopsy and BAL assessments, differences in treatment group medians and bootstrap CIs were used to summarize treatment differences. The bootstrap CIs were based on 1000 samples (each with replacement) from each treatment group and the 2.5% and 97.5% quantiles of the resulting sampling distribution.¹⁷ Analysis of covariance was used to summarize treatment differences for reticular basement membrane thickness. Within- and between-treatment group differences were evaluated with the Wilcoxon signed-rank and rank sum tests, respectively.

Safety was assessed on the basis of clinical adverse events and exacerbations. The incidence of asthma exacerbations was compared between treatments with the Cochran-Mantel-Haenszel test, controlling for investigator.¹⁸

RESULTS

Subjects

A total of 244 subjects were initially screened, and 88 fulfilled the inclusion criteria, were randomized to therapy with an ICS alone or an ICS plus a LABA, and underwent bronchoscopy. Baseline demographic and clinical indices were similar between treatment groups (Table I) and were similar to those of subjects previously reported by Busse et al.¹¹ Additional demographic information is provided in Table E1 (see the Online Repository at www.jacionline.org).

Physiologic and clinical indices

The self-reported diary card measures of lung function, asthma symptoms, and rescue albuterol use were similar for both the 250 µg of FP twice daily group and the 100/50 µg of FSC twice daily group at baseline. Statistically significant within-treatment group changes from baseline are shown in Table I. There were greater increases in morning PEF with 100/50 µg of FSC twice daily when compared with 250 µg of FP twice daily (44.6 and 31.3 L/min, respectively), as was the case with evening PEF (45.0 and 24.0 L/min, respectively), but differences between these increases were not statistically significant. For the other measures (FEV₁, percentage of symptom-free and rescue-free days, albuterol use, asthma symptom scores, and nighttime awakenings), there were improvements in both

groups over the 24 weeks that were not statistically different when comparing the 2 treatment arms (Table I).

Immunohistochemical analyses

Seventy-five matched-pair biopsy samples were available for analysis (36 for the 100/50 µg of FSC twice daily group and 39 for the 250 µg of FP twice daily group). Thirteen subjects did not have adequate paired specimens for analysis.

No significant differences were found between the 2 treatment groups at baseline for outcome parameters in the biopsy specimens. The most prevalent cell type in both groups in the submucosa was neutrophils, and although the counts were slightly, albeit not statistically significantly, higher in the FP group (median, 71.97 vs 53.13 cells/mm²) at baseline, this between-group difference did not seem to be important because it continued to be observed after 24 weeks of treatment (73.38 vs 58.71 cells/mm²). The median number of eosinophils per square millimeter of submucosa (the primary end point) was 0.86 (interquartile range [IQR], 0.00-3.63) in the FSC group and 1.18 (IQR, 0.00-3.22) in the FP group at baseline (*P* = .719) and 0.99 (IQR, 0.00-3.03) and 0.00 (IQR, 0.00-1.78), respectively, after treatment (*P* = .297). This change in eosinophil counts in the FP group was also not significant in a within-group comparison (*P* = .051). No eosinophils were detected per millimeter length of epithelium for the majority of subjects (Table II).

After the 24-week randomized treatment period, there were modest decreases in submucosal mast cell numbers from baseline in both treatment groups. Although the median decrease from baseline to end point in submucosal mast cells was significant for the 250 µg of FP twice daily group (32.11 cells/mm² down to 18.43 cells/mm², *P* = .004), there were no significant differences in changes at week 24 in the number of mast cells per square millimeter of submucosa or millimeter of intact epithelium between subjects randomized to receive 100/50 µg of FSC twice daily or those randomized to receive 250 µg of FP twice daily (*P* ≥ .199). There were also no other significant within-treatment changes in the epithelial or submucosal counts of neutrophils, total T cells (CD3⁺), CD4⁺ or CD8⁺ T-cell subsets, or activated CD25⁺ T cells (Table III).

For thickness of the lamina reticularis, the mean ± SE pretreatment values were 11.16 ± 0.43 µm and 10.37 ± 0.29 µm (*P* = .122) and 10.17 ± 0.47 and 10.53 ± 0.29 after treatment (*P* = .101) in the FSC and FP groups, respectively.

BAL analyses

Paired BALF samples were available for 76 of 87 subjects. The missing samples were either due to study withdrawal, missing cell counts, or insufficient BAL volumes. The median values for absolute eosinophil counts and percentages of total cells were 0.04 × 10⁶ cells/mL (IQR, 0.01-0.16 × 10⁶ cells/mL) and 0.80% (IQR, 0.20%-1.95%) in FSC-treated subjects and 0.04 × 10⁶ cells/mL

TABLE I. Baseline characteristics and change from baseline at end point (weeks 1-24) in patient-recorded clinical data from the biopsy study population

	FSC, 100/50 µg BID (n = 40)	FP, 250 µg BID (n = 48)	Treatment difference*† (95% CI)
Mean age, y (SD)	34.3 (11.3)	35.3 (14.6)	—
Male/female, %	43/58	46/54	—
White, %	95	92	—
FEV ₁ , L			
Baseline	3.02 (0.10)	2.91 (0.10)	0.06 (−0.02 to 0.15)
End point	3.03 (0.11)	2.87 (0.11)	
Mean change	0.04 (0.03)	−0.02 (0.03)	
FEV ₁ , % predicted			
Baseline	84.5 (1.50)	81.2 (1.36)	1.82 (−0.59 to 4.23)
End point	84.6 (1.52)	80.3 (1.60)	
Mean change	0.84 (0.93)	−0.72 (0.78)	
Morning PEF, L/min			
Baseline	498 (20.5)	470 (19.1)	7.4 (−24.7 to 39.5)
End point	539 (24.5)	503 (20.8)	
Mean change	44.6 (9.4)‡	31.3 (10.9)‡	
Morning PEF, % predicted			
Baseline	105.2 (3.8)	97.8 (3.1)	0.7 (−6.3 to 7.8)
End point	114.2 (4.6)	105.5 (4.0)	
Mean change	9.4 (1.9)‡	7.2 (2.5)‡	
Evening PEF, L/min			
Baseline	499 (20.0)	478 (18.6)	16.1 (−18.1 to 50.3)
End point	541 (24.0)	504 (21.1)	
Mean change	45.0 (9.9)‡	24.0 (11.1)‡	
Evening PEF, % predicted			
Baseline	105.3 (3.5)	99.6 (2.9)	2.4 (−5.1 to 9.9)
End point	114.4 (4.3)	106.0 (4.2)	
Mean change	9.4 (2.0)‡	5.8 (2.6)‡	
Percentage of symptom-free days			
Baseline	47 (6.4)	47 (5.7)	0.3 (−14.8 to 15.4)
End point	63.8 (7.2)	59.4 (6.3)	
Mean change	16.1 (5.1)‡	12.1 (5.1)‡	
Daily asthma symptom score§			
Baseline	0.85 (0.14)	0.87 (0.12)	0.05 (−0.26 to 0.36)
End point	0.60 (0.15)	0.71 (0.14)	
Mean change	−0.23 (0.09)‡	−0.20 (0.12)	
Albuterol use (puffs/24 h)			
Baseline	0.7 (0.11)	1.1 (0.21)	−0.21 (−0.72 to 0.30)
End point	0.42 (0.10)	0.80 (0.22)	
Mean change	−0.24 (0.11)‡	−0.29 (0.23)	
Percentage of rescue-free days			
Baseline	56 (6.6)	55 (5.6)	5.4 (−9.1 to 20.0)
End point	72.8 (6.1)	67.2 (5.8)	
Mean change	16.9 (5.8)‡	12.0 (4.6)‡	

SEs are shown in parentheses unless otherwise indicated.

BID, Twice daily.

*Differences between treatments for all end points were within the 95% CIs for equivalence.

†No statistically significant differences between treatment groups.

‡Statistically significant ($P \leq .037$) differences within treatment groups.

§Scale: 0 = no symptoms; 5 = severe symptoms.

(IQR, 0.02-0.08 $\times 10^6$ cells/mL) and 0.60% (IQR, 0.40%-1.20%) in FP-treated subjects, respectively. Neither the absolute nor the relative eosinophil counts changed significantly from baseline in either treatment group. There were also no differences between the treatment groups in the absolute or relative counts of BAL macrophages, lymphocytes, or neutrophils. Finally, there were no differences

between treatment groups in BAL concentrations of GM-CSF, IL-8, or ECP (Table IV).

Safety

Adverse events. Both of the treatments were well tolerated, and the incidence of common adverse events was similar in the 2 treatment groups. Adverse events

TABLE II. Summary of total eosinophils per area (in square millimeters) of tissue in submucosa and total eosinophils per millimeter length of epithelium

	FSC, 100/50 µg BID	FP, 250 µg BID	Treatment difference*	95% CI*†
Eosinophils (cells/mm ² submucosa)				
Baseline (n)	40	46		
Median (IQR‡)	0.86 (0.00-3.63)	1.18 (0.00-3.22)		
Mean (SE)	3.62 (1.00)	3.45 (0.97)		
End point (n)	36	40		
Median (IQR‡)	0.99 (0.00-3.03)	0.00 (0.00-1.78)	0.14	−0.64 to 1.67
Mean (SE)	3.59 (1.09)	2.48 (0.96)		
Eosinophils (cells/mm epithelium)				
Baseline (n)	34	39		
Median (IQR‡)	0.00 (0.00-0.00)	0.00 (0.00-0.00)		
Mean (SE)	0.03 (0.03)	0.06 (0.05)		
End point (n)	28	30		
Median (IQR‡)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	0.00	0.00 to 0.00
Mean (SE)	0.11 (0.11)	0.06 (0.06)		

BID, Twice daily.

*Estimate of the difference in median changes from baseline to end point; 95% CI for the difference in median changes.

†No statistically significant differences between treatment groups ($P \geq .297$).

‡Denotes IQR (25th-75th percentiles); no statistically significant changes within treatment groups ($P \geq .051$).

TABLE III. Summary of mast cells, neutrophils, and CD3⁺, CD4⁺, CD8⁺, and CD25⁺ lymphocytes in the submucosa (median [IQR*])

Cell	FSC, 100/50 µg BID		FP, 250 µg BID		Treatment difference	95% CI†‡
	Baseline (n = 40)	End point (n = 36)	Baseline (n = 46)	End point (n = 40)		
Mast cells	36.50 (20.14-52.87)	29.07 (15.98-63.33)	32.11 (15.32-59.52)	18.43§ (6.14-33.22)	7.98	−3.64 to 24.26
Mean (SE)	44.04 (5.48)	37.35 (4.73)	42.73 (5.52)	28.81 (5.02)		
Neutrophils	53.13 (37.98-81.12)	58.71 (38.39-118.88)	71.97 (48.68-133.21)	73.38 (41.04-125.11)	5.26	−19.9 to 48.56
Mean (SE)	76.68 (11.53)	85.86 (12.14)	112.27 (16.84)	86.46 (10.13)		
CD3 ⁺	47.78 (30.17-86.73)	51.18 (30.67-77.14)	65.46 (27.45-103.28)	45.31 (17.96-112.55)	9.55	−20.2 to 38.77
Mean (SE)	67.67 (8.96)	76.63 (14.03)	85.27 (12.65)	68.33 (9.72)		
CD4 ⁺	28.53 (16.58-47.58)	35.05 (19.57-65.63)	34.06 (17.20-80.33)	24.94 (13.51-67.45)	15.01	−5.41 to 31.06
Mean (SE)	39.72 (5.59)	53.92 (10.79)	52.24 (7.33)	42.29 (7.20)		
CD8 ⁺	8.72 (4.29-19.47)	8.65 (3.06-19.09)	11.87 (5.00-20.31)	7.17 (0.68-25.58)	−0.11	−4.94 to 6.66
Mean (SE)	17.17 (3.41)	19.91 (6.10)	19.05 (3.84)	15.32 (2.85)		
CD25 ⁺	0.00 (0.00-0.98)	0.19 (0.00-1.12)	0.21 (0.00-1.00)	0.00 (0.00-0.58)	0.00	0.00 to 0.51
Mean (SE)	0.88 (0.37)	0.99 (0.35)	0.75 (0.19)	0.41 (0.11)		

BID, Twice daily.

*Denotes IQR (25th-75th percentiles).

†Estimate of the difference in median changes from baseline to end point; 95% CI for the difference in median changes.

‡No statistically significant differences between treatment groups ($P \geq .065$).

§Statistically significant ($P = .004$) changes within treatment groups; otherwise $P \geq .064$.

considered by the investigators to be related to study treatment occurred in 6 (15%) and 8 (17%) subjects treated with 100/50 µg of FSC twice daily and 250 µg of FP twice daily, respectively. No adverse event occurred in more than one patient in each treatment group. The pharmacologically predictable adverse events included oral candidiasis (1 patient in each group), palpitation (1 patient in the FP group), and headache (1 patient in the FSC group). There were no serious drug-related adverse events during treatment. Two subjects receiving 100/50 µg of FSC twice daily withdrew from the study because of cancer and

pregnancy, respectively. No complications were observed from the bronchoscopy performed before randomization or at week 24 after randomization.

Exacerbations. An exacerbation was defined as worsening asthma requiring treatment beyond the blinded study drug and supplemental albuterol use. The number of subjects who experienced asthma exacerbations was low and similar in the 2 treatment groups. Five (13%) and 9 (19%) subjects treated with 100/50 µg of FSC twice daily and 250 µg of FP twice daily, respectively, experienced exacerbations. Two of the subjects receiving

TABLE IV. Summary of BAL cell differentials (in percentages) and inflammatory mediators (median [IQR*])

Cell or mediator	FSC, 100/50 µg BID		FP, 250 µg twice daily		Treatment difference	95% CI†‡
	Baseline (n = 40)	End point (n = 36)	Baseline (n = 47)	End point (n = 39)		
Eosinophils (%)	0.80 (0.20-1.95)	0.60 (0.30-1.95)	0.60 (0.40-1.20)	0.40 (0.20-1.00)	0.40	−0.20 to 0.70
Mean (SE)	2.03 (0.68)	2.40 (0.98)	1.83 (0.69)	1.59 (0.68)		
Macrophages (%)	86.75 (83.70-91.85)	87.95 (80.75-92.70)	88.80 (81.70-92.10)	90.00 (81.50-92.60)	1.05	−2.60 to 2.75
Mean (SE)	86.49 (1.06)	86.30 (1.42)	85.94 (1.38)	86.12 (1.67)		
Neutrophils (%)	1.00 (0.55-2.30)	1.50 (0.60-3.25)	1.50 (0.70-2.50)	1.40 (0.50-2.70)	0.45	−0.20 to 1.05
Mean (SE)	2.00 (0.37)	2.70 (0.54)	1.78 (0.21)	4.00 (1.48)		
Lymphocytes (%)	8.80 (6.55-12.80)	7.65 (4.60-11.00)	7.70 (4.80-13.50)	6.30 (3.90-11.80)	0.59	−1.65 to 2.83
Mean (SE)	9.50 (0.74)	8.59 (0.78)	10.46 (1.23)	8.27 (0.97)		
Cell or mediator	Baseline (n = 39)	End point (n = 33)	Baseline (n = 44)	End point (n = 37)	Treatment difference	95% CI†‡
GM-CSF (pg/mL)	1.00 (0.50-1.64)	1.16 (0.62-1.66)	1.19§ (0.48-2.57)	1.05§ (0.34-1.95)	0.34	−0.12 to 0.75
Mean (SE)	1.39 (0.27)	1.38 (0.25)	1.80 (0.29)	1.22 (0.17)		
IL-8 (pg/mL)	12.46 (4.90-24.15)	14.83 (9.57-22.55)	19.29 (8.17-36.62)	22.12 (13.04-38.94)	1.60	−8.05 to 8.57
Mean (SE)	20.60 (4.14)	22.81 (3.93)	30.30 (5.11)	77.58 (30.76)		
Cell or mediator	Baseline (n = 22)	End point (n = 18)	Baseline (n = 27)	End point (n = 22)	Treatment difference	95% CI†‡
ECP (µg/mL)	0.00 (0.00-0.35)	0.00 (0.00-0.80)	0.00 (0.00-0.35)	0.15 (0.00-0.84)	0.00	−0.36 to 0.00
Mean (SE)	0.56 (0.38)	0.44 (0.17)	0.34 (0.18)	0.72 (0.31)		

BID, Twice daily.

*Denotes IQR (25th-75th percentiles).

†Estimate of the difference in median changes from baseline to end point; 95% CI for the difference in median changes.

‡No statistically significant differences between treatment groups ($P \geq .083$).§Statistically significant ($P = .011$) changes within treatment groups; otherwise $P \geq .066$.

250 µg of FP twice daily withdrew from the study because of exacerbations. The remaining subjects who experienced exacerbations were successfully treated with prednisone and completed the study.

DISCUSSION

This study demonstrated that in patients with moderate asthma, clinical asthma control and control of the underlying airway inflammation and airway remodeling were maintained when patients were treated with 100/50 µg of FSC or a higher dose of an ICS (250 µg of FP). This study therefore provides direct evidence that reduction of ICSs, when done in conjunction with the introduction of a LABA, is safe and does not lead to worsening of airway inflammation.

The clinical results of the present study confirm the recent results from another study with identical trial design but without biopsy and BAL,¹¹ showing that lung function, asthma symptoms, and rescue albuterol use are all preserved when switching from a medium-dose ICS to a low-dose ICS plus a LABA. In addition, a recent meta-analysis of 9 studies has shown significant reductions in the incidence of asthma exacerbations with corresponding significant improvements in lung function and asthma symptoms when using a lower dose of ICS plus salmeterol compared with a higher-dose ICS alone.¹⁹

When selecting subjects for this study, attention was given to excluding subjects with COPD. We are confident

that this was achieved by using stringent exclusion clinical criteria and the presence of symptoms typical of asthma. Current smokers and those with a significant smoking history were excluded. Given the age of the patients in this study and the limited smoking history, it is unlikely that patients with COPD were enrolled. BAL neutrophil counts, although higher than eosinophil counts, were in keeping with previous reports in healthy nonsmokers and were not as high as normally reported in patients with COPD.²⁰⁻²² It should also be noted that the presence of neutrophilia in this population would not be entirely surprising because neutrophilia is a well-recognized feature of airway inflammation in patients with more severe disease that could be related, at least in part, to the effect of corticosteroid therapy.^{23,24}

A critical question when using a lower-dose ICS plus a LABA versus higher doses of an ICS alone has been whether such a treatment paradigm unmasks underlying airway inflammation, allows further airway remodeling to occur, or both.

In this study eosinophil numbers in both the bronchial mucosa and BALF, which sample the proximal and distal airway compartments, respectively, were unchanged when patients were switched to a lower dose of FP plus salmeterol. This is in keeping with observations made by Kips et al,²⁵ who showed no difference in sputum eosinophils over a period of 1 year in patients taking a lower dose of budesonide plus formoterol when compared with those taking higher-dose budesonide alone. Eosinophils were chosen as the primary outcome of the bronchial biopsy

and BAL analyses because they are viewed as an important marker of asthmatic inflammation and are broadly correlated with disease severity.^{26,27} Although the role of eosinophils in asthma pathogenesis has been questioned recently,²⁸ these cells have regained importance with the demonstration that treatment of asthma aimed at maintaining sputum eosinophil counts within the normal range is superior to a strategy based on symptom control alone.²⁹ Studies have demonstrated that excessive ICS reduction in patients receiving a LABA results in an increase in sputum eosinophils and clinical exacerbations.⁸ Thus eosinophils either directly contribute to, or are a marker of, a more complex process that leads to asthma exacerbations.

Other inflammatory indices in the current study, including the mucosal numbers of neutrophils, mast cells, and T cells, were not significantly different between treatment groups at the end of the treatment period. All of these findings, together with the finding that the lamina reticularis thickness, an indicator of airway remodeling, was also unchanged, strongly suggests that the use of a LABA to reduce the dose of ICS caused neither an increase in airway inflammation nor a progression of remodeling during the course of this study.

The current study has potential limitations. First, because the dose of ICS given at baseline might have been in excess of that required to control clinical activity and airway inflammation, it cannot be excluded that a lower ICS dose alone would have been adequate to control inflammation. However, it is important to note that worsening of symptoms and increases in indices of airway inflammation are coupled, as has been shown by Green et al²⁹ and Lazarus et al,¹⁰ and there is nothing markedly different in the design of this study to suggest that this would not have been the case were we able to sample the airways at the time of exacerbation. Nonetheless, we believed that another bronchoscopy during asthma deterioration was both unsafe and likely to cause significant discomfort to volunteers. Therefore we chose to address this issue in the current study by including a run-in period to establish the minimum effective ICS dose required to achieve asthma control, as assessed on the basis of clinical outcome measures. Specifically, during the first run-in period, potential subjects were required to demonstrate clinical deteriorations in the following: FEV₁, nighttime awakenings, or albuterol use when receiving open-label 100 µg of FP twice daily (run-in period 2). Having demonstrated deterioration in asthma control, patients received open-label 250 µg of FP twice daily to re-establish asthma control. Because asthma symptoms and short-acting β₂-agonist use persisted during the use of open-label 250 µg of FP twice daily (run-in period 3), it is unlikely that asthma and inflammation were overtreated at the time of the first bronchoscopy.

The second potential limitation is the duration of the study. A study that is not sufficiently long might fail to detect the emergence of airway inflammation or changes in airway remodeling that could require longer periods of time to become manifest. However, studies by Green

et al,¹⁰ McIvor et al,⁸ and Lazarus et al²⁹ have shown that the presence of inadequate anti-inflammatory control results in the emergence of airway inflammation within 4 to 7 weeks, suggesting that the current study duration was sufficient to detect emerging inflammation, were it to have occurred. Nevertheless, it is plausible that the detection of long-term changes associated with airway remodeling might require studies of longer duration.

In the current study we did not identify cellular mechanisms that might point to synergy at the cell level, but it is known that ICSs and LABAs target different underlying components of asthma, primarily inflammation and smooth muscle dysfunction, respectively. There is growing evidence that the clinical benefits of ICSs and LABAs result from their complementary modes of action, which result in benefits that are at a minimum additive or possibly synergistic.³⁰ Notably, LABAs have complementary effects on the ICS receptor, and ICSs have complementary effects on the β-receptor. *In vitro* studies have shown that β₂-agonists promote the ligand-independent activation of the glucocorticoid receptor.^{31,32} Alternatively, clinical doses of glucocorticoids have been shown to increase the number of β₂-agonist receptors.^{33,34} These proposed complementary effects of ICSs and LABAs might explain why lower doses of ICSs might control asthma symptoms and inflammation in the presence of salmeterol, but these effects remain to be demonstrated in other *in vivo* studies, such as ours.

In conclusion, this study demonstrates that control of both asthma and airway inflammation is maintained over 24 weeks when patients requiring a medium dose of an ICS are switched to a lower dose of an ICS in combination with a LABA. These data confirm that treatment with a low dose of an ICS plus a LABA is as effective as a higher dose of ICS in controlling airway inflammation and is ICS sparing. Together with the clinical efficacy studies, this study shows that switching from a moderate-dose ICS to therapy with a lower-dose ICS plus salmeterol is an efficacious and safe therapeutic option.

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REFERENCES

- Barnes PJ, Pedersen S, Busse WW. Efficacy and safety of inhaled corticosteroids. New developments. *Am J Respir Crit Care Med* 1998; 157(suppl):1S-53S.
- Umland SP, Schleimer RP, Johnson SL. Review of the molecular and cellular mechanisms of action of glucocorticoids for use in asthma. *Pulm Pharmacol Ther* 2002;15:35-50.
- Wallin A, Sue-Chu M, Bjerner L, Ward J, Sandstrom T, Lindberg A, et al. Effect of inhaled fluticasone with and without salmeterol on airway inflammation in asthma. *J Allergy Clin Immunol* 2003;112:72-8.
- Laitinen LA, Laitinen A, Haahtela T. A comparative study of the effects of an inhaled corticosteroid, budesonide, and a beta₂-agonist, terbutaline, on airway inflammation in newly diagnosed asthma: a randomized, double-blind, parallel-group controlled trial. *J Allergy Clin Immunol* 1992; 90:32-42.
- Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, Walters EH. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309-16.
- Wong CA, Walsh LJ, Smith CJP, Wisniewski AF, Lewis SA, Hubbard R, et al. Inhaled corticosteroid use and bone-mineral density in patients with asthma. *Lancet* 2000;355:1399-403.
- Cumming RG, Mitchell P, Leeder SR. Use of inhaled corticosteroid and the risk of cataracts. *N Engl J Med* 1997;337:8-14.
- McIvor RA, Pizzichini E, Turner MO, Hussack P, Hargreave FE, Sears MR. Potential masking effects of salmeterol on airway inflammation in asthma. *Am J Respir Crit Care Med* 1998;158:924-30.
- Lemanske RF, Sorkness CA, Mauger EA, Lazarus SC, Boushey HA, Fahy JV, et al. Inhaled corticosteroid reduction and elimination in patients with persistent asthma receiving salmeterol. *JAMA* 2001;285:2594-603.
- Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanske RF Jr, Sorkness CA, et al. Long-acting beta₂-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. *JAMA* 2001;285:2583-93.
- Busse W, Koenig SM, Oppenheimer J, Sahn SA, Yancey SW, Reilly D, et al. Steroid-sparing effects of FP 100mcg and salmeterol 50mcg BID administered in a single product in patients previously controlled with FP 250mcg BID. *J Allergy Clin Immunol* 2003;111:57-65.
- 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.
- Britten KM, Howarth PH, Roche WR. Immunohistochemistry on resin sections: a comparison of resin embedding techniques for small mucosal biopsies. *Biotech Histochem* 1993;68:271-80.
- Sullivan P, Stephens D, Ansari T, Costello J, Jeffery P. Variation in measurements of basement membrane thickness and inflammatory cell number in bronchial biopsies. *Eur Respir J* 1998;12:811-5.
- Kraft M, Martin RJ, Lazarus SC, Fahy JV, Boushey HA, Lemanske RF, et al. Airway tissue mast cells in persistent asthma. Predictor of treatment failure when patients discontinue inhaled corticosteroids. *Chest* 2003;124:42-50.
- Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Oliveri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 1997;111:852-7.
- Efron B, Tibshirani RJ. An introduction to the bootstrap. London: Chapman and Hall; 1993.
- Mantel N, Haenszel W. Statistical aspects of the analysis of data from retrospective studies of disease. *J Natl Cancer Inst* 1959;22:719-48.
- Shrewsbury S, Pyke S, Britton M. Meta-analysis of increased dose of inhaled steroid or addition of salmeterol in symptomatic asthma (MIASMA). *BMJ* 2000;320:1368-73.
- Anonymous. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis, and selected comparison groups. The BAL Cooperative Group Steering Committee. *Am Rev Respir Dis* 1990; 141(suppl):S169-202.
- Hunninghake GW, Crystal RG. Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers. *Am Rev Respir Dis* 1983;128:833-6.
- Thompson AB, Daughton D, Robbins RA, Ghafouri MA, Oehlerking M, Rennard SI. Intraluminal airway inflammation in chronic bronchitis. Characterization and correlation with clinical parameters. *Am Rev Respir Dis* 1998;140:1527-37.
- Anonymous. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. European Network for Understanding Mechanisms of Severe Asthma. *Eur Respir J* 2003;22:470-7.
- Wenzel SE, Szefer SJ, Leung DY, Sloan SI, Rex MD, Martin RJ. Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 1997; 156:737-43.
- Kips JC, O'Connor BJ, Inman MD, Svensson K, Pauwels RA, O'Byrne PM. A long-term study of the antiinflammatory effect of low-dose budesonide plus formoterol versus high-dose budesonide in asthma. *Am J Respir Crit Care Med* 2000;161:996-1001.
- Bentley AM, Menz G, Storz C, Robinson DS, Bradley B, Jeffery PK, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. *Am Rev Respir Dis* 1992; 146:500-6.
- Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 2000;161:9-16.
- Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144-8.
- Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715-21.
- Nelson HS, Chapman KR, Pyke SD, Johnson M, Pritchard JN. Enhanced synergy between fluticasone propionate and salmeterol inhaled from a single inhaler versus separate inhalers. *J Allergy Clin Immunol* 2003; 112:29-36.
- Johnson M. Combination therapy for asthma: Complementary effects of long-acting beta₂-agonists and corticosteroids. *Curr Allergy Clin Immunol* 2002;15:16-22.
- Eickelberg O, Roth M, Lox R, Bruce V, Rudiger J, Johnson M, Block LH. Ligand-independent activation of the glucocorticoid receptor by beta₂-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 1999;274:1005-10.
- Baraniuk JN, Ali M, Brody D, Maniscalco J, Gaumont E, Fitzgerald T, et al. Glucocorticoids induce beta₂-adrenergic receptor function in human nasal mucosa. *Am J Respir Crit Care Med* 1997;155:704-10.
- Roth M, Johnson PR, Rudiger JJ, King GG, Ge Q, Burgess JK, et al. Interaction between glucocorticoids and beta₂ agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet* 2002;360:1293-9.