Changes in sputum counts and airway hyperresponsiveness after budesonide: Monitoring anti-inflammatory response on the basis of surrogate markers of airway inflammation

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Background: Airway hyperresponsiveness (AHR) to pharmacologic stimuli and sputum eosinophils might be useful in the individual adjustment of long-term asthma management. However, it is not clear whether inhaled glucocorticosteroids (GCSs) provide greater protection against specific surrogate markers of airways inflammation than other means. In addition, detailed longitudinal assessment of changes in airway response with inhaled GCSs has never been carried out.
Objectives: We compared changes in AHR to inhaled methacholine and adenosine 5′-monophosphate (AMP) after budesonide treatment in a randomized, double-blind, placebo-controlled, crossover study of patients with mild-to-moderate asthma. Subsequently, we undertook a separate study to examine the time course of the changes in AHR in more detail and the changes in sputum cell counts in relation to budesonide treatment.
Methods: In the phase 1 of the study, patients undertook bronchial provocation studies with increasing doubling concentrations of methacholine (0.06 to 16 mg/mL) and AMP (3.125 to 800 mg/mL) before and after budesonide 0.8 mg/daily for 3 weeks. The bronchial responses to the inhaled agonists were expressed as the provocative concentration causing a 20% decline in FEV_1 (PC_{20}). In phase 2 of the study, patients attended the laboratory on 12 separate occasions to investigate changes in PC_{20} methacholine, PC_{20} AMP, and sputum cell counts before, during, and after withdrawal of therapy with inhaled budesonide 0.8 mg/daily for 6 weeks.
Results: Budesonide treatment for 3 weeks significantly attenuated the constrictor response by 0.8 ± 0.3 doubling doses for methacholine and by 2.6 ± 0.5 doubling doses for AMP. These changes were significantly different from each other ($P = .003$). Significant variation in PC_{20} methacholine ($P < .05$) value, PC_{20} AMP ($P < .001$) value, percentage of sputum eosinophils ($P < .001$), and percentage of sputum epithelial cells ($P < .001$) were observed throughout the longitudinal assessment of changes in airway response to budesonide. Compared with the other surrogate markers, PC_{20} AMP appears to be useful in promptly detecting early inflammatory changes of the asthmatic airways; a significant change of 1.6 ± 0.3, 2.2 ± 0.3, and 2.8 ± 0.3 doubling doses of PC_{20} AMP was observed at 1, 4, and 6 weeks, respectively, in the course of budesonide treatment.
Conclusions: The present findings underline the exquisite selectivity of diverse surrogate markers of airway inflammation in response to inhaled budesonide. When compared with that to the other markers, AHR to inhaled AMP is an early and sensitive indicator of the beneficial anti-inflammatory effects of topical GCSs. (J Allergy Clin Immunol 2002;110:855-61.)

Key words: *Bronchial hyperresponsiveness, adenosine challenge, induced sputum, budesonide*

Asthma is a chronic inflammatory condition of the airways characterized by bronchial obstruction and airway hyperresponsiveness (AHR) to pharmacologic and physical stimuli.[1] Sensible use of prophylactic agents with topical anti-inflammatory activity, such as inhaled glucocorticosteroids (GCSs), is the cornerstone of asthma management.[1] Inhaled GCSs have been shown to reduce symptoms and the frequency of asthma exacerbations, to improve lung function, and to ensure a better long-term outcome.[2]

Current Global Initiative for Asthma guidelines recognize the need for developing noninvasive tests of asthma airway inflammation for use in monitoring the disorder's activity and the anti-inflammatory effects of asthma therapy.[1] To this end, a number of noninvasive diagnostic tests have been evaluated, including bronchoprovocation with direct and indirect stimuli and sputum induction. Indirect noninvasive assessment of airway inflammation by measurement of AHR[3-6] and sputum eosinophilia[6-9] has proved to be sensitive to inhaled GCSs. The relationship between AHR and airway eosinophilic inflammation remains controversial, and there is little evidence that lung function and symptoms are associated with inflammatory changes.[10-12] Specifically, we[13] and other investigators[14] have shown that AHR to inhaled adenosine (which mainly reflects a selective interaction with activated airway mast cells[15]) is more strongly associated with sputum eosinophilia when compared with nonspecific stimuli, such as histamine or methacholine. The view that AHR to

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TABLE I. Details of patients' characteristics

Patient no.	Sex	Age (y)	Baseline FEV_1 (% predicted)	Atopy	PC_{20} Meth (mg/mL)	PC_{20} AMP (mg/mL)
1	M	24	84	D	0.15	10.9
2	F	22	75	W	0.40	4.6
3	M	19	77	G-D	0.25	6.5
4	M	23	80	G	1.34	21.6
5	F	25	84	D	0.22	44.7
6	M	24	73	D	0.55	32.2
7	M	27	70	D	0.30	11.4
8	M	35	80	G-T-W	0.86	32.1
9	M	51	71	G	1.17	8.9
10	F	24	85	G	0.37	4.5
11	F	29	75	G-D	0.11	17.0
12	F	48	89	G-T	0.67	15.7
13	F	36	75	D	0.71	9.7
14	M	31	100	W	4.72	105.7
15	F	35	109	G	1.02	40.7
16	F	23	107	G-W	1.96	125.9
17	M	31	76	G-D-W	1.09	44.2
18	M	33	78	G	1.45	22.0
19	M	30	99	G-D	0.41	5.0
20	M	20	81	D	0.75	13.3
21	M	36	98	W-T	0.69	22.6
Mean (± SEM)		29.8 (1.9)	84.1 (2.6)	—	—	—
Geometric mean (range)		—	—	—	0.62 (0.11-4.72)	18.2 (4.5-125.9)

Meth, Methacholine; *AMP,* adenosine 5′-monophosphate; *D,* positive skin test result with *Dermatophagoides pteronyssinus* and/or *Dermatophagoides farinae*; *G,* positive skin test result with mixed grass pollen; *T,* positive skin test result with mixed tree pollen; *W,* positive skin test result with mixed weed pollen.

Abbreviations used
AHR: Airway hyperresponsiveness
AMP: Adenosine 5′-monophosphate
GCS: Glucocorticosteroid

adenosine might be used as a closer surrogate marker of airway inflammation than AHR to histamine or methacholine has been addressed in a number of clinical studies in which airways responsiveness to adenosine monophosphate was better at discriminating changes in airway reactivity with topical GCSs when compared with that to histamine or methacholine.[16-18] Taken together, these findings appear to support the concept that AHR to inhaled adenosine, sputum eosinophils, or both might be useful in the individual adjustment of long-term asthma management.

However, it is not clear whether inhaled GCSs provide greater attenuation against specific surrogate markers of airways inflammation than other means. We have therefore compared changes in AHR to inhaled methacholine and adenosine 5′-monophosphate (AMP) and in sputum cell counts after inhaled budesonide in a randomized, double-blind, crossover study of patients with mild asthma. Because all published studies describe changes in airway response after inhaled GCS only at a single time point and little is known of the change in AHR with time, we also examined the time course of change in surrogate markers of airways inflammation before, during, and after withdrawal of therapy with inhaled budesonide.

METHODS

Patients

A total of 26 nonsmoking subjects with mild asthma were recruited for the study. Definition of disease severity was based on the criteria set in the Global Initiative for Asthma guidelines.[1] Five patients had to be excluded from analysis (2 failed to attend the scheduled visits in the course of their second treatment period, 1 was noncompliant with medication use, and 2 failed to produce adequate sputum samples in their subsequent visits), hence the results are based on a total of 21 patients (Table I). All were atopic, as defined by a positive skin prick test reaction (>3-mm wheal response) to one or more of 7 common aeroallergens. Inclusion criteria comprised stable asthma (having had no exacerbation or respiratory tract infection in the previous 2 months), baseline FEV_1 of greater than 70% of predicted values, documented AHR to inhaled AMP, and ability to produce sputum on induction with hypertonic saline. Patients had never used inhaled corticosteroids before or had stopped their use at least 2 months before entry into the study. Throughout the study, only short-acting inhaled β_2-adrenoreceptor agonists were allowed for relief of symptoms but were withheld for 8 hours before each visit to the laboratory. Antihistamines were not taken at least 3 days before each visit. The protocol was reviewed by the local hospital's ethics committee, and written informed consent was obtained for each patient.

Study design

The study consisted of 2 distinct phases, and all visits to the laboratory took place outside the pollen season.

Phase 1. Eleven patients with asthma (patients 1-11) were randomized by using computer-generated numbers into a double-blind, placebo-controlled, crossover study with 0.8 mg/d budesonide or placebo, each for 21 days, separated by a washout period of at least 3 weeks. Patients attended the laboratory on 2 consecutive mornings before and after each treatment period to undertake concentration-response studies with inhaled methacholine and AMP. Each patient was challenged with methacholine on the first morning, followed by AMP challenge the next day. The order of inhalation challenges was identical for all patients throughout the study. Treatment was commenced immediately after completion of AMP challenge and continued up to the second (AMP) challenge morning.

Phase 2. Ten asthmatic patients (patients 12-21) attended the laboratory on 12 separate occasions for up to 10 weeks to investi-

gate the time course of changes in AHR to inhaled methacholine and AMP and in sputum cell counts before (baseline time point at 0 weeks), during (time points at 1, 4, and 6 weeks), and after withdrawal (time points at 7 and 10 weeks) of therapy with 0.8 mg/d inhaled budesonide. Patients attended the laboratory on 2 consecutive mornings at each time point to undertake concentration-response studies with methacholine and AMP and sputum induction. Each patient was challenged with methacholine, followed by AMP challenge 2 to 3 hours later on the first morning, which was followed by sputum induction the next day. Sputum induction and challenge procedures used were identical to those described in phase 1 of the study. This order was kept identical for all patients throughout the 10 weeks of the study. Treatment was commenced immediately after completion of the first sputum induction and continued for up to 6 weeks.

Bronchoprovocation testing with inhaled methacholine and AMP

AHR was evaluated by means of methacholine and AMP bronchial challenge, as described previously.[19] In brief, methacholine (Lofarma, Milan, Italy) and AMP (Sigma Chemical Co, St Louis, Mo) were dissolved in PBS (pH 7.4) and normal saline, respectively, to produce increasing doubling concentrations (0.06-16 mg/mL for methacholine; 3.125-800 mg/mL for AMP). Solutions were administered as aerosols generated from a starting volume of 3 mL in a disposable Inspiron Minineb (C.R. Bard International, Sunderland, United Kingdom) driven by compressed air at 8 L/min. Patients inhaled increasing doubling concentrations of agonist in 5 breaths from functional residual capacity to total lung capacity through a mouthpiece, and FEV_1 was measured at 1 and 3 minutes after each administration. The challenges were stopped when a decrease of 20% in FEV_1 had been achieved or when the maximum concentration of agonist had been inhaled. The bronchial responses to the inhaled agonists were expressed as the provocative concentration causing a 20% decrease in FEV_1 (PC_{20}) value, which was calculated by means of linear interpolation from the concentration-response curve constructed on a logarithmic scale.

Sputum induction and processing

Induction was performed according to our previously published method.[13] Briefly, participants inhaled hypertonic saline (4.5%) for up to 5 consecutive 5-minute periods until an adequate volume of sputum was collected. Sputum plugs were transferred into 50-mL polypropylene tubes (Becton Dickinson, Abingdon, United Kingdom) and treated with an equal weight of 0.01 mol/L dithioerythritol (Fluka, Gillingham, Dorset, United Kingdom). Specimens were then vortexed for 10 seconds, rocked for 30 minutes, and then filtered through a 70-μm strainer (Becton Dickinson) before centrifuging at 400*g* for 10 minutes at 4°C. Cell pellets were resuspended in 1 mL of PBS without Ca^{2+} and Mg^{2+}, and viable cells were counted in a hemocytometer. Only samples in which squamous cells comprised less than 30% of total cells were considered satisfactory for analysis. Differential cell counts were carried out on coded cytospin slides stained with May-Grunwald-Giemsa by one experienced investigator on 600 nonsquamous cells. For epithelial cell immunochemical staining, cytospin preparations were fixed in cold methanol for 10 minutes and incubated with a mouse anti-human mAb against cytokeratin proteins 5, 6, 8, 17, 18, and 19 (Dako, Wycombe, United Kingdom). Binding was detected by using peroxidase-conjugated secondary antibodies and visualized with diaminobenzidine. Experiments included control slides lacking primary antibody, as well as an isotype-matched antibody. The average results of differential cell counts were taken and expressed as percentages of the number of total nonsquamous cells.

Statistical analysis

All the variables in the sputum that were not normally distributed were expressed as medians with interquartile ranges. PC_{20} values were logarithmically transformed to normalize their distribution and expressed as geometric means with ranges. For all nonparametric data, differences between treatment groups were compared by using the Friedman test, followed by the Wilcoxon matched-pairs signed-rank test where appropriate. The difference (after minus before) in log PC_{20} values for methacholine and AMP after active and placebo treatments were compared by using ANOVA for multiple comparisons, and the change was assessed by using the least-significant-difference test to allow for multiple comparisons. Pretreatment and posttreatment spirometry readings were expressed as means ± SEM and compared by using paired *t* tests. The protective effect of treatment on provocation responses was calculated by comparing the difference in (after minus before) log PC_{20} after active and placebo treatments in each individual subject and expressed in terms of mean ± SEM doubling dilutions to compare variations in AHR to different agonists; paired *t* tests were used for statistical comparisons. The Spearman correlation test was used to analyze relationships between sputum variables and PC_{20} AMP and PC_{20} methacholine.

A 2-tailed *P* value of less than .05 was considered to indicate statistical significance. All analyses were performed with the Statistical Package for Social Sciences (SASS, Chicago, Ill) for Windows version 10.0.

RESULTS

Phase 1

FEV_1 values at baseline were not significantly different between treatment periods. Treatment with budesonide caused a small but statistically significant 5.4% increase in mean FEV_1 ($P = .037$), whereas placebo did not produce significant changes.

PC_{20} FEV_1 values at baseline were not significantly different between treatment periods. Budesonide treatment for 3 weeks significantly attenuated the constrictor response to both agonists, with their PC_{20} FEV_1 values increasing from 0.45 mg/mL (range, 0.13-1.25 mg/mL) to 0.85 mg/mL (range, 0.45-1.62 mg/mL; $P < .05$, after placebo vs after budesonide treatment) and from 14.3 mg/mL (range, 6.0-46.9 mg/mL) to 92.4 mg/mL (range, 15.3-340.6 mg/mL; $P < .001$, after placebo vs after budesonide treatment) for methacholine and AMP, respectively. In contrast, PC_{20} FEV_1 values before and after placebo treatment were not significantly different.

When changes in the protective effect of inhaled budesonide on provocation responses were expressed as doubling dilutions, a mean ± SEM protection of 0.8 ± 0.3 doubling doses against methacholine and of 2.6 ± 0.5 doubling doses against AMP were reported (Fig 1). These changes were significantly different from each other ($P = .003$).

Phase 2

Budesonide caused a small but statistically significant 7.7% increase in mean FEV_1 ($P = .014$, baseline vs 6 weeks after budesonide treatment) by the end of the 6-week treatment period.

Significant variation in PC_{20} methacholine ($P < .05$) and AMP ($P < .001$) values were observed throughout the study (Fig 2). Comparisons with baseline PC_{20} metha-

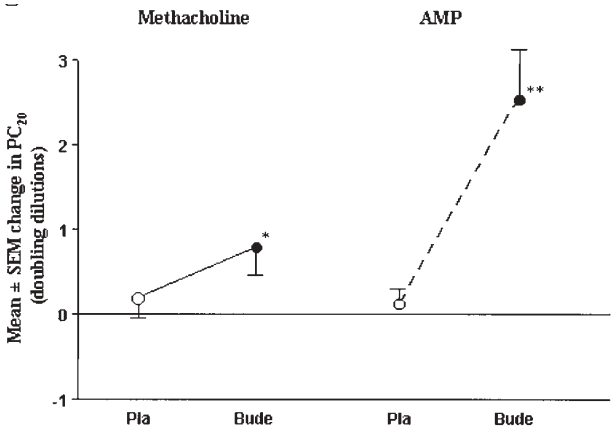

FIG 1. Changes in PC_{20} methacholine and PC_{20} AMP values expressed as mean ± SEM doubling dilutions after each 3-week treatment period with placebo (*Pla*; *open circles*) and budesonide (*Bude*; *filled circles*). $*P < .05$ and $**P < .001$ (after placebo vs after budesonide treatment).

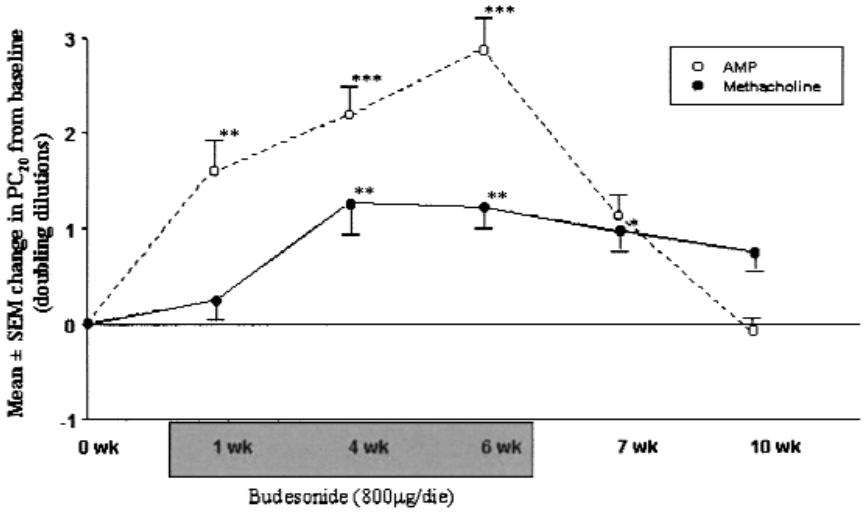

FIG 2. Comparative changes in PC_{20} methacholine *(filled circles)* and PC_{20} AMP *(open circles)* values expressed as mean ± SEM doubling dilutions before, during, and after discontinuation of budesonide treatment. $*P < .05$, $**P < .01$, and $***P < .001$ (after treatment vs baseline).

choline value throughout the 10-week study period showed a significant difference at 4 weeks ($P < .01$) and 6 weeks ($P < .01$) after budesonide treatment and at week 7 ($P < .05$), 1 week after discontinuation of budesonide; the geometric mean PC_{20} methacholine value at baseline increased from 1.04 mg/mL (range, 0.41-4.72 mg/mL) to 2.48 mg/mL (range, 0.88-5.97 mg/mL) at 4 weeks to 2.45 mg/mL (range, 1.11-7.96 mg/mL) at 6 weeks and to 2.05 mg/mL (range, 0.79-5.45 mg/mL) at 7 weeks (Table II). When changes in PC_{20} methacholine values over time were expressed as doubling dilutions, a mean ± SEM change of 0.3 ± 0.2, 1.3 ± 0.3, 1.2 ± 0.3, 1.0 ± 0.2, and 0.7 ± 0.2 doubling doses were shown at 1, 4, 6, 7, and 10 weeks, respectively (Fig 2). Comparisons with baseline PC_{20} AMP values throughout the 10-week study period showed a significant difference at 1 week ($P < .01$), 4 weeks ($P < .001$), and 6 weeks ($P < .001$) after budesonide treatment, whereas no significant change was observed at any time point after treatment discontinuation; the geometric mean PC_{20} AMP value at baseline increased from 25.6 mg/mL (range, 9.7-125.9 mg/mL) to 76.1 mg/mL (range, 11.3-215.3 mg/mL) at 1 week, to 117.0 mg/mL (range, 47.9-259.7 mg/mL) at 4 weeks, and to 179.5 mg/mL (range, 39.9-546.5 mg/mL at 6 weeks (Table II). When changes in PC_{20} AMP values over time were expressed as doubling dilutions, mean ± SEM changes of 1.6 ± 0.3, 2.2 ± 0.3, 2.8 ± 0.3, 1.1 ± 0.2, and –0.1 ± 0.1 doubling doses were shown at 1, 4, 6, 7, and 10 weeks, respectively (Fig 2).

Significant variation in the percentage of sputum eosinophils ($P < .001$) and epithelial cells ($P < .001$) were observed throughout the study (Fig 3). Percentages of eosinophils in induced sputum were significantly reduced at 4 weeks ($P < .01$) and 6 weeks ($P < .01$) after budesonide treatment and remained significantly lowered at both time points after discontinuation of budesonide ($P < .01$ at 7 weeks and $P < .05$ at 10 weeks); the median percentage of sputum eosinophils of 10.5% (interquartile range, 6.8%-

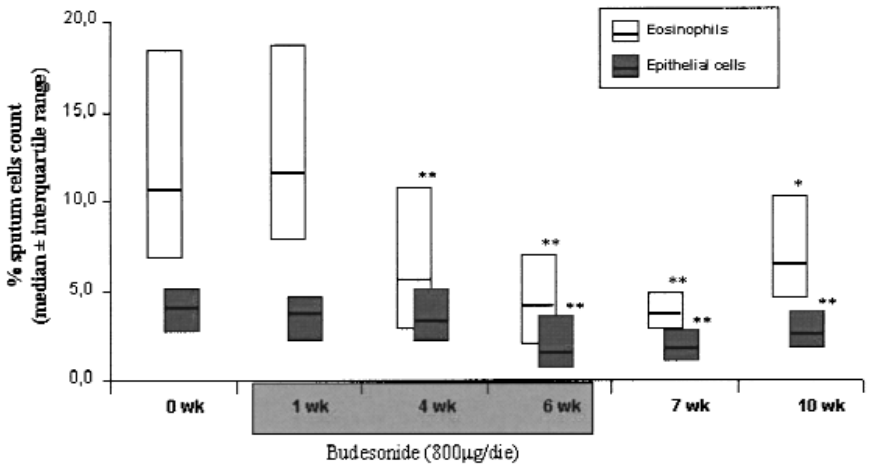

FIG 3. Comparative changes in median (± interquartile range) percentage of eosinophil *(open boxes)* and epithelial cell *(filled boxes)* counts in the sputum before, during, and after discontinuation of budesonide treatment. Medians are shown as *thick horizontal bars*. *$P < .05$ and **$P < .01$ (after treatment vs baseline).

TABLE II. Time course of changes in airway hyperresponsiveness to methacholine and AMP before, during, and after withdrawal of budesonide treatment

Patient no. Baseline	1 wk Post-bude	4 wk Post-bude	6 wk Post-bude	7 wk	10 wk	
PC_{20} AMP (mg/mL)						
1	15.7	72.7	66.6	101.7	36.6	16.9
2	9.7	11.3	47.9	39.9	12.1	7.9
3	105.7	215.3	187.1	291.0	80.3	87.6
4	40.7	202.6	239.9	251.7	124.8	39.9
5	125.9	188.1	259.7	300.9	228.0	119.7
6	44.2	81.8	147.0	546.5	85.6	47.1
7	22	58.0	94.4	185.8	52.1	14.4
8	5	40.6	55.9	94.5	15.7	5.9
9	13.3	31.2	73.0	125.6	33.8	13
10	22.6	161.3	229.1	322.7	111.3	29.1
Geometric mean (range)	25.6 (9.7-125.9)	76.1 (11.3-215.3)	117.0 (47.9-259.7)	179.5 (39.9-546.5)	55.3 (12.1-228.0)	24.7 (5.9-119.7)
PC_{20} methacholine (mg/mL)						
1	0.67	0.44	0.97	1.28	0.79	0.86
2	0.71	1.83	2.70	1.57	1.88	1.02
3	4.72	5.80	5.72	7.96	5.45	6.21
4	1.02	0.75	1.66	2.40	1.98	1.59
5	1.96	2.74	5.97	2.93	3.53	2.76
6	1.09	1.64	4.19	3.90	1.64	1.04
7	1.45	1.00	0.88	1.80	1.72	2.22
8	0.41	0.75	1.96	1.11	1.44	0.98
9	0.75	1.55	3.22	5.93	3.25	2.91
10	0.69	0.49	2.58	1.51	1.73	1.57
Geometric mean (range)	1.04 (0.41-4.72)	1.25 (0.44-5.80)	2.48 (0.88-5.97)	2.45 (1.11-7.96)	2.05 (0.79-5.45)	1.74 (0.86-6.21)

Post-bude, After withdrawal of budesonide treatment.

18.5%) at baseline was reduced to 5.5% (interquartile range, 2.8%-10.5%) at 4 weeks, to 4.0% (interquartile range, 2.0%-7.0%) at 6 weeks, to 3.5% (interquartile range, 2.8%-5.0%) at 7 weeks, and to 6.5% (interquartile range, 4.8%-10.5%) at 10 weeks (Fig 3). In contrast, percentages of epithelial cells in the sputum became significantly reduced only at 6 weeks ($P < .01$) after budesonide treatment but remained significantly lower at both time points after discontinuation of budesonide ($P < .01$ at 7 weeks and $P < .01$ at 10 weeks); the median percentage of sputum epithelial cells of 4.0% (interquartile range, 2.9%-5.2%) at baseline was reduced to 1.5% (interquartile range, 0.9%-3.5%) at 6 weeks, to 1.8% (interquartile range, 1.0%-2.7%) at 7 weeks, and to 2.5% (interquartile range, 1.9%-3.6%) at 10 weeks (Fig 3).

At baseline, we observed a significant negative correlation between sputum eosinophils and PC_{20} AMP value ($R_s = –0.72$, $P = .03$) but not PC_{20} methacholine value ($R_s = –0.38$, $P = .31$). The association between sputum eosinophils and AMP PC_{20} value was maintained during budesonide treatment at 4 weeks ($R_s = –0.68$, $P = .04$) and at 6 weeks ($R_s = –0.77$, $P = .02$).

No significant variation in the proportion of sputum neutrophils, macrophages, and lymphocytes was observed throughout the study.

DISCUSSION

Our study set out to investigate the effect of treatment with budesonide on specific surrogate markers of airways inflammation in patients with mild-to-moderate allergic asthma. Budesonide provided a significantly greater reduction in AHR to inhaled AMP than to methacholine. Longitudinal assessment of changes in surrogate markers of airways inflammation in the course of budesonide treatment showed important variation in PC_{20} methacholine and PC_{20} AMP values, with a significant reduction in the percentage of eosinophil and epithelial cell counts in the sputum. However, when compared with the other markers, AHR to inhaled AMP promptly detected inflammatory changes of the asthmatic airways as early as the first week of budesonide treatment. Moreover, PC_{20} AMP values returned to near-baseline levels as early as the first week of treatment discontinuation. These findings underline the exquisite sensitivity of adenosine in response to a topical GCS.

Three weeks' treatment with budesonide caused a dissimilar degree of reduction in the response to methacholine and AMP, displacing their dose-response curve to the right by 0.8 and 2.6 doubling dilutions, respectively. These effects of 0.8 mg/d inhaled budesonide for 3 weeks are compatible with previous data by O'Connor et al,[16] who observed a reduction of 1.2 and 2.9 doubling dilutions in the response to methacholine and AMP after 2 weeks of treatment with 1.6 mg/d budesonide. Similar results have been obtained in earlier randomized controlled studies, in which AMP provocation better discriminated changes in airway reactivity with inhaled fluticasone[17] and ciclesonide[18] when compared with histamine or methacholine. The recent study by van den Berge et al[20] clearly emphasizes that, when compared with PC_{20} methacholine, PC_{20} AMP better reflects the reduction in airway inflammation observed with GCS. Thus short-term treatment with topical GCSs has a significant but small effect on AHR to direct stimuli, whereas it markedly reduces AHR to AMP. The explanation for the greater protective effect of budesonide on AMP over methacholine is not known but must relate to their different mechanism or mechanisms of action.

The mechanisms of adenosine-induced bronchoconstriction appear to involve stimulation of specific adenosine A_{2B} receptors on airway mast cells, with subsequent release of preformed and newly formed contractile mediators.[21] In addition to affecting smooth muscle responsiveness, budesonide might inhibit the airway response to inhaled AMP either at a cellular level by reducing the number and function of airway mast cells or at a molecular level by downregulating the activity of A_{2B} receptors. Marked reductions in the number of mast cells have been observed in the bronchial mucosa of patients with asthma after regular treatment with inhaled beclomethasone dipropionate[22] or budesonide[23] as a result of reduction in the expression of stem cell factor (a growth factor that promotes mast cell chemotaxis and differentiation).[24] A significant decrease in the concentration of tryptase, a marker of mast cell activation in the sputum of asthmatic subjects has been recently reported after budesonide treatment.[25] Although limited, there is some evidence that GCSs might also downregulate the activity of adenosine A_{2B} receptors.[26] Whatever the mechanism accounting for the greater protective effect of budesonide on AMP over methacholine, it is apparent that responsiveness of the asthmatic airways to inhaled AMP is a sensitive indicator of underlying inflammation. This view is also supported by the observation that in asthmatic children allergen avoidance at high altitude resulted in a pronounced improvement in AHR to AMP, although not to methacholine.[27] Conversely, in individuals with allergic rhinitis, deteriorations in AHR during the onset of the pollen season were consistently detected with AMP challenge but not with methacholine challenge.[28]

We have extended our initial observation of a greater protective effect of budesonide on AMP over methacholine by examining in more detail the change in AHR to both agonists and in sputum cell counts before, during, and after withdrawal of budesonide treatment. We confirmed the greater protection against PC_{20} AMP than PC_{20} methacholine and showed a reduction in both sputum eosinophil and epithelial cell counts in the course of treatment. The effect of inhaled budesonide on sputum eosinophils and epithelial cells was sustained well beyond treatment discontinuation for up to 4 weeks, whereas PC_{20} AMP values reverted to near-baseline levels by the first week of budesonide withdrawal. These findings are in agreement with previous data on asthmatic patients showing that inhaled budesonide efficiently reduces the percentage of sputum eosinophils.[6,29] The recent work of in't Veen et al[30] investigating the effect of steroid tapering on sputum eosinophils has shown a significant increase in their numbers at 5 weeks. This is in accordance with our findings of an apparent trend toward an increase in sputum eosinophil counts 4 weeks after budesonide discontinuation.

To the best of our knowledge, this is the first study that has examined the effect of inhaled GCSs on sputum epithelial cells. A significant reduction was observed at 6 weeks after budesonide treatment and was maintained for at least 4 weeks after treatment discontinuation. This finding was not unexpected because inhaled budesonide is reported to enhance epithelial integrity in vivo.[31] This could be due to an inhibition of epithelial cell apoptosis induced by Fas ligation.[32] Moreover, whereas a period of antigen avoidance at high altitude is associated with a reduction in sputum epithelial cells of children with allergic asthma,[33] the bronchial epithelial cell count in the sputum of allergic individuals is invariably increased during the peak of a pollen season.[28]

It is of interest that when comparing the 3 markers, AHR to inhaled AMP promptly detected inflammatory changes of the asthmatic airways as early as the first week of treatment with budesonide, whereas changes in AHR to methacholine and in sputum cell counts could be observed only by the fourth week of treatment. Kanniess et al[25] have also shown that although there was no signif-

icant effect of low-dose inhaled budesonide for 2 weeks with regard to sputum eosinophils, a significant change in PC_{20} AMP values was already apparent in patients with mild asthma. That AHR to inhaled AMP more closely reflects reduction in airway inflammation after inhaled corticosteroids than inflammatory markers in induced sputum has been also substantiated in the study by Taylor et al,[18] in which the effect of different doses of inhaled ciclesonide on lung function, AHR to AMP, and sputum eosinophils was assessed. There was a dose-dependent decrease in AHR to AMP when comparing 100 and 400 μg of ciclesonide, whereas the reduction in the percentage of sputum eosinophils did not differ between doses. Taken together, these findings validate the exquisite sensitivity of AMP responsiveness to topical GCSs.

We suggest that these markers might be useful in the individual adjustment of long-term asthma management. In particular, when compared with the other markers, AHR to inhaled AMP is an early and sensitive indicator of the anti-inflammatory effects of topical GCSs. Therefore monitoring of AHR to inhaled AMP might be one of the most accurate guides to monitor inhaled GCS requirements in asthma and to establish the appropriate dose needed to control airway inflammation. This, however, requires further studies involving a larger number of patients.

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