

A comparative study of the effects of an inhaled corticosteroid, budesonide, and a β_2 -agonist, terbutaline, on airway inflammation in newly diagnosed asthma: A randomized, double-blind, parallel-group controlled trial

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We compared the effect of an inhaled corticosteroid, budesonide, and an inhaled β_2 -agonist, terbutaline, on clinical symptoms, lung function, and airway inflammation in 14 adult patients with newly diagnosed asthma. The study was conducted as a randomized, double-blind, parallel-group trial. Seven patients inhaled 600 μ g, twice daily, of budesonide, the other seven patients inhaled 375 μ g, twice daily, of terbutaline via identical metered-dose inhalers with a spacer. Bronchial biopsy specimens, obtained before randomization and after 3 months of treatment, were analyzed by electron microscopy. Both groups improved clinically budesonide was more effective than terbutaline in improving morning and evening peak expiratory flow rates, as well as bronchial responsiveness to inhaled histamine. Treatment with budesonide was accompanied by increased numbers of ciliated airway cells and intraepithelial nerves and fewer inflammatory cells, including eosinophils, especially in the epithelium, these changes were not observed in specimens from terbutaline-treated patients. We conclude that, in contrast to inhaled terbutaline, inhaled budesonide improved lung function and bronchial hyperreactivity in adult subjects with asthma treated for 3 months and that this corticosteroid was more effective in ameliorating abnormalities of the bronchial epithelium and decreasing inflammation in the airways. (J ALLERGY CLIN IMMUNOL 1992;90:32-42.)

Key words: Asthma, airway inflammation, budesonide, bronchial reactivity, electron microscopy, eosinophils, terbutaline

Morphologic studies of bronchial biopsy specimens have contributed to the present understanding of asthma as an inflammatory disease of the airways.¹⁻⁵ The airway mucosa reveals a distinctive cellular pic-

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Abbreviations used

c/g Index:	Ratio of ciliated cells to goblet cells
c/o Index:	Ratio of ciliated cells to both goblet cells and other epithelial cells
b.i.d.:	Twice daily
MDI:	Metered-dose inhaler
PC ₁₅ :	Provocative concentration of histamine causing a 15% decrease in FEV ₁
PEFR:	Peak expiratory flow rate

ture with chronic inflammation and concomitant vascular changes.⁶ The importance of this inflammatory component in the course of the disease, until recently underestimated, is now receiving a great deal of attention.^{7, 8} As suggested by both these morphologic changes and the clinical efficacy of inhaled glucocorticoids, an inflammatory process is present in the

airways even at an early stage of the disease.^{9, 10} It has been suggested that drugs that act mainly on the smooth muscle component of airway obstruction may mask the inflammatory component and that priority should be given to treating the inflammation.⁸

The effect of an inhaled corticosteroid on the cellular aspects of airway inflammation has not been investigated directly in controlled studies. However, in three uncontrolled studies, inhaled corticosteroids reduced the number of inflammatory cells in the airways.¹¹⁻¹³ According to one case report, treatment with an inhaled corticosteroid for 4 months that resulted in improvement in clinical status and airway hyperresponsiveness was accompanied by a decrease in the number of eosinophils in the airway epithelium.¹⁴ Inhaled corticosteroid has also been demonstrated to decrease eosinophil cationic protein in the bronchoalveolar lavage fluid of patients with asthma.¹⁵

We therefore undertook a controlled trial in which bronchial biopsy specimens were obtained from patients with newly diagnosed asthma, who were then treated for 3 months with either an inhaled corticosteroid, budesonide, or with an inhaled β_2 -agonist, terbutaline. Each drug was administered as the only regular medication. We studied the effect of this treatment on asthma symptoms, lung function, (including bronchial responsiveness to histamine), the structure of the bronchial epithelium, and the number of inflammatory cells in a mucosa.

MATERIAL AND METHODS

Patients

Fourteen adult patients (four men and 10 women) with newly diagnosed asthma and no previous regular treatment were selected for enrollment in this study. The diagnosis of asthma was based on published criteria.¹⁶ The mean age of the patients was 42 years (range, 21 to 59 years), mean height, 164 cm (range, 146 to 182 cm), mean weight, 67 kg (range, 50 to 100 kg), and mean duration of asthma, 7.4 months (range, 2 to 12 months). Four patients in each group were atopic, as determined by their clinical history and by two or more positive skin prick tests for 12 common inhalant allergens. The mean blood eosinophil counts ($\pm$ SE) in the steroid-treated and terbutaline-treated groups were $0.211 \pm 0.199 \times 10^9$ cells per liter and $0.808 \pm 1.737 \times 10^9$ cells per liter, respectively. None of the subjects were smokers or had had a respiratory tract infection during the 2 months preceding their enrollment in the study. The two groups were comparable with respect to all demographic data and to the duration of their asthma. Informed consent was obtained from all participants, and the study was approved by the Finnish Health Authorities and by the Ethics Committee of the Kanta-Häme Central Hospital.

Study design

The study was conducted as a randomized, double-blind, parallel-group trial. Subjects entered the study at staggered

intervals during a 12-month period. The study started with a 6-week baseline period to ensure that patients were duly instructed and were following the protocol and that their prebiopsy treatment was adequately regulated. At the end of that period, patients were randomized into one of the two groups. Treatment was started immediately and continued for 3 months. Patients returned to the clinic during the sixth and twelfth weeks of treatment for pulmonary function tests and, at the twelfth visit, for a bronchial biopsy. None of the investigators was aware of the patient's treatment until all the study data had been collected and analyzed.

Treatment protocol

Treatment for all patients during the baseline period consisted of 375 μ g (three puffs) of terbutaline (Bricanyl; AB Astra, Södertälje, Sweden), inhaled b.i.d. through an MDI. After the baseline period, subjects were randomly assigned to receive either budesonide (Pulmicort; AB Astra) at a dose of 600 μ g (three puffs) b.i.d., or 375 μ g of terbutaline, b.i.d. Both drugs were inhaled through identical-appearing MDIs with a large volume spacer (Nebuhaler; AB Astra). Each subject also received a 250 μ g per dose of terbutaline MDI to be used when it was necessary. Seven subjects were assigned to each group. After randomization, any subject having a serious exacerbation of asthma symptoms received, in addition, a 6-day course of oral prednisolone, administered in decreasing doses (30, 25, 20, 15, 10, and 5 mg/day).

Clinical and pulmonary function data

Throughout the entire study period, patients recorded their asthma symptoms (dyspnea, cough, sputum production, and effect on daily activities) and assigned a single score from 0 to 10 to each 24-hour period. Patients also recorded their use of any supplemental medication and any adverse reactions that occurred. In addition, patients measured their PEF with a mini-Wright peak flow meter (Clement Clarke, Int. Ltd., London, England), every morning and evening before taking the treatment. Measurements of FVC and FEV₁ with a Vitalograph dynamic wedge-bellows spirometer (Vitalograph Ltd., Buckingham, U.K.) and tests for bronchial responsiveness to histamine were performed in the clinic on the first study visit, again 2 weeks later, and then at the beginning of active treatment and at 6-week intervals during the treatment period. Patients were instructed to take a dose of the study drug in the evening before each appointment and then to use no supplemental medication during the 6 hours before the appointment. Bronchial reactivity was assessed with a buffered histamine di-phosphate solution administered via a DeVilbiss No. 40 nebulizer (DeVilbiss Co., Somerset, Pa.) operated at an airflow of 5 L/min, as described previously.⁸ Internebulizer variability was tested, and only nebulizers with an output of 0.125 to 0.25 ml/min were used. The same nebulizer or, if this were necessary, a nebulizer with exactly the same output, was used for each patient. Solutions containing 1, 2, 4, 8, 16, and 32 mg/ml of histamine were used. Each test began with 10 tidal-breath inhalations of a saline solution. The test was continued with inhalations of succes-

sively higher concentrations of histamine at 5-minute intervals until a PC_{15} from the postsaline control value was obtained. FEV_1 was measured 1½ minutes after each challenge. The best value of at least two measurements with a difference of <4% was recorded. This value was compared with the value obtained after saline inhalation. Challenge testing was stopped when the FEV_1 fell by 15% or more. The concentration of histamine that produced a PC_{15} was calculated by interpolation and called the "provocative concentration." If the FEV_1 decreased <15%, even when the highest concentration of histamine was used, then the PC_{15} was considered to be 64 mg/ml.

Bronchial biopsies

Bronchial biopsy specimens were obtained from each patient before randomization and again after 3 months of receiving active treatment through a rigid tube bronchoscope (Storz 10318C, Storz GmbH and Co, Tuttlingen, Germany, diameter 7.5 mm, length 43 cm) with the patient under local anesthesia. Patients were also administered 10 mg of diazepam (Diazemuls; AB Astra, 5 mg/ml), intravenously, for sedation before the procedure. The bronchoscope was inserted into the right main bronchus and biopsy specimens from the airway mucosa were obtained through the scope with a forceps (Storz 1037L). The epithelium at the biopsy sites had not been touched by the scope before the biopsy specimens were obtained. Biopsy specimens were obtained from two different airway levels: (1) inside the right upper lobe bronchus and (2) at the opening of the right middle lobe. The specimens were fixed and embedded in Epon as previously described.¹ All the specimens were coded and examined by the electron microscopist (A. L.) without knowledge of the patient, the timing of the biopsy, or the treatment taken. Sections were first prepared and observed under a light microscope. The area of bronchial epithelium in the section demonstrating no artifactual damage caused by manipulation by the forceps was chosen for further preparation and analysis by electron microscopy. A total of 54 biopsy specimens were available for examination, only the right upper lobe specimens before and after treatment being available from one patient. Transmission electron microscopic examinations were performed with a Jeol (Jeol Ltd., Akishima-Shi, Japan) JEM 1200 EX microscope at the Department of Electron Microscopy, University of Helsinki. Thin sections were examined on Slot 1 by 2 mm grids without grid bars (LKB, Wallac, Sweden), as described previously.¹⁴ A cover made with pioloform resin (Polaron Instruments, Inc. [Bio-Rad, Microscience Div.], Cambridge, Mass.) was used as a support for the section on the grid. This method allows examination of larger uniform areas of the specimen (1000 by 2000 µm) than is possible with normal 200 to 300 mesh grids. The whole thin section was photographed in the electron microscope at an original magnification of $\times 800$. Adjacent electron micrographs with a final magnification of $\times 2000$ were combined to form a montage of the thin section. All individual inflammatory cells were photographed at $\times 5000$ magnification, and each individual cell was subsequently identified in the montage on the basis of its morphology.¹⁷ The montage was charted

semiautomatically into a Compaq Deskpro 386 computer (Compaq Computer Corp., Houston, Texas) via a 42 by 60 inch Kurta IS/THREE digitizing tablet (Kurta Corp., Phoenix, Ariz.) with a pointing device. Formation of the image-picture and data processing of a number of cells per area were performed by means of the AUTOCAD graphics program (Autodesk, Inc.). The cell population (inserted by type) of both the epithelium and the lamina propria was determined per square millimeter. To evaluate the epithelial structure quantitatively, the numbers of ciliated, goblet, and other epithelial cells were counted at the point at which they reached the airway lumen. We calculated c/g index and the c/o index. In addition, the number of intraepithelial nerves was analyzed as previously described.¹⁸

Statistical analysis

The point of reference for the three lung-function variables, FVC, FEV_1 and PC_{15} , was the date of randomization. Logarithmic values for PC_{15} were used in the analyses, and the results were expressed as dose steps, for example, step 0, 1 mg/ml; step 1, 2 mg/ml; etc. The values for the four variables recorded in the patients' diaries, the asthma symptom scores, the number of puffs of supplemental terbutaline, and morning and evening PEFs, were first reduced to averages for the 6-week pretreatment period and for the concluding 3-week periods during active treatment. The pretreatment average was used as the reference value. Intra- and intergroup comparisons of lung function variables were performed with Student's paired and unpaired *t* tests, respectively. Analysis of the biopsy data included the following steps. First, changes after treatment were computed for each variable. These changes were then tested for zero mean values in all patients within a treatment group and between treatment groups. Standard nonparametric tests (signed Wilcoxon's and Wilcoxon's rank-sum test) were used to avoid assumptions about distributions.

RESULTS

Clinical and pulmonary function data

The mean asthma symptom score for all 14 patients during the pretreatment period was 3.2 (range, 0.08 to 6.6). During the same period, the mean value of the morning PEF (as percent of the predicted normal value) was 94% (73% to 111%) and the mean value of the evening PEF was 99% (range, 82% to 118%). At the visit before randomization, the mean FVC was 91% (range, 68% to 113%) of the predicted normal value, the mean FEV_1 was 89% (range, 62% to 104%) of the predicted normal value, and the mean FEV_1 , as a percentage of FVC (FEV_1/FVC percent), was 81% (range, 68% to 95%). The mean PC_{15} as determined in the histamine provocation test was 2.9 mg/ml (range, 0.9 to 11.1 mg/ml).

During the treatment period, asthma symptom scores and the use of supplemental medication improved more in the budesonide-treated group than in the terbutaline-treated group, but the intergroup dif-

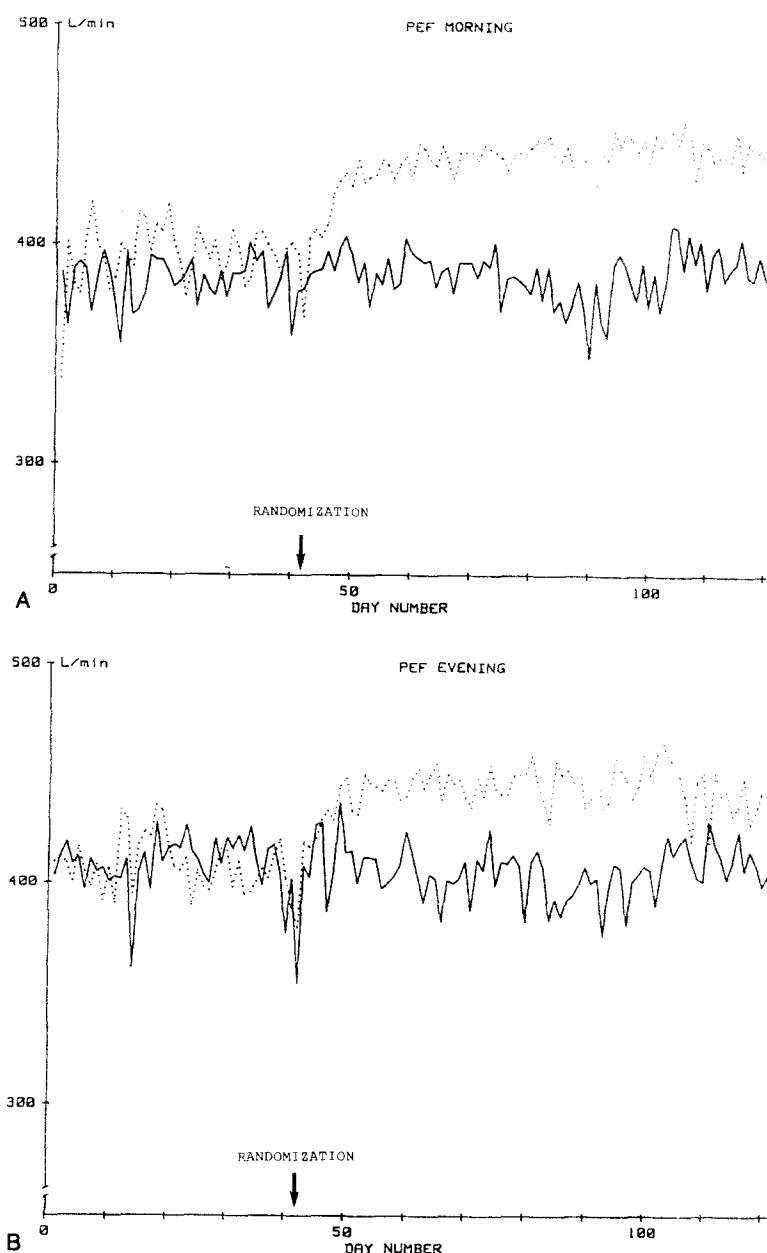


Fig. 1. Three months of budesonide treatment. **A**, Significantly increased morning ($p < 0.001$) PEFR values (---). **B**, Significantly increased evening PEFR values (---). Terbutaline treatment had no effect (—).

ferences were not statistically significant. Peak flow measurements in the morning and evening increased significantly ($p < 0.001$) in the budesonide-treated group but not in the terbutaline-treated group (Fig 1, A and B). When the two groups were compared, both variables were significant, in favor of the budesonide-treated group ($p < 0.001$ for the morning PEFR and $p < 0.05$ for the evening PEFR). FVC (percent of predicted) increased in both treatment groups but did not differ significantly between the groups. FEV₁ (per-

cent predicted) increased significantly in the budesonide-treated group ($p < 0.05$) but not in the terbutaline-treated group, although, again, there was no significant difference between the two groups. FEV₁/FVC percent did not change significantly within the groups, whereas mean values did decrease in the terbutaline-treated group, making the intergroup difference significant, in favor of budesonide ($p < 0.05$). In the results of histamine-challenge tests, when the difference in PC₁₅ between visits 3

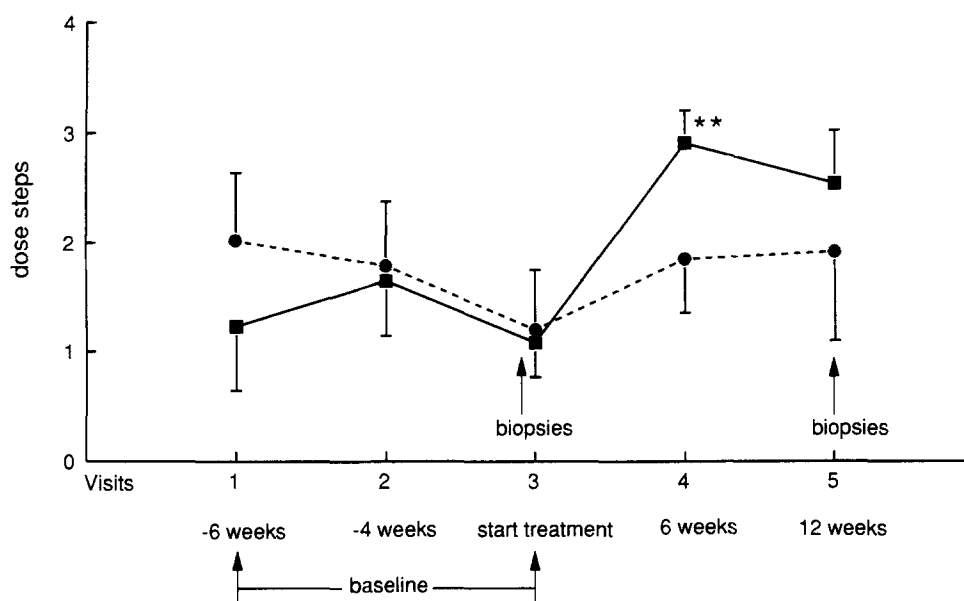


Fig. 2. PC₁₅ (mean ± SE) in tests of bronchial responsiveness in the treatment groups. Mean difference between budesonide (■—■) and terbutaline (●—●) treatments in degree of change is significant (** $p = 0.002$) at 6 weeks after randomization, but only almost significant ($p = 0.06$) at the 12-week visit. PC₁₅ dose steps represent histamine concentrations of 1, 2, 4, 8, 16, and 32 mg/ml.

(start of treatment) and 4 (after 6 weeks of treatment) and between visits 3 and 5 (after 12 weeks of treatment) was analyzed for each patient and computed for the two groups, a significant increase was noted for the budesonide-treated group between visits 3 and 4 ($p = 0.002$); the increase between visits 3 and 5 almost reached significance ($p = 0.06$) (Fig. 2).

Morphologic observations

With the aid of the electron microscope, the mean length of the epithelium in the airway of the 49 specimens examined (of a total of 54 biopsy specimens available), corresponding to the length of the basement membrane, measured 730 μm (range, 300 to 1160 μm). The lamina propria under the corresponding epithelium and the basement membrane had a mean depth of 200 μm (range, 100 to 270 μm).

When the pretreatment specimens were scrutinized, we found no difference ($p > 0.05$) in the number of cells in biopsy specimens from the upper and middle lobe bronchi; therefore, data from these two sources were pooled. In specimens from both groups of patients, the airway epithelium contained fewer ciliated cells relative to goblet cells and other undifferentiated epithelium cells in comparison with specimens obtained at the end of the treatment period. With the first biopsy, the mean c/g index was 2.3 (range, 0 to 9.1) in the budesonide-treated group and 2.1 (range, 0 to 4.8) in the terbutaline-treated group. The mean

c/o indexes for the two groups were 1.1 (range, 0 to 4) and 1.6 (range, 0 to 4), respectively. Goblet cell hyperplasia, with or without any ciliated cells, dominated the epithelial structural changes. Occasional foci of epithelial metaplasia were also observed.

After treatment with inhaled budesonide, the structure of the airway epithelium was improved, as presented by significant increases in the c/g index (6.1; range, 1.3 to 14.2; $p < 0.05$) and the c/o index (4.4; range, 0.3 to 10.9; $p < 0.01$) with ciliated cells predominating in the epithelium in all but three specimens (c/g and c/o indexes ≥ 3). In contrast, after terbutaline treatment, there was no improvement in the airway epithelial structure, and both the c/g index (3.2; range, 0.4 to 10) and the c/o index (2.1; range, 0.3 to 6) were similar to those obtained with specimens obtained before treatment. Moreover, after budesonide treatment, the number of intraepithelial nerves increased significantly from a mean value of 10.6 to 38 nerves per square millimeter ($p < 0.05$), whereas after terbutaline therapy, no increase in the number of intraepithelial nerves was observed. The difference between the two groups was therefore statistically significant ($p < 0.05$).

After treatment with inhaled budesonide, the total number of cells (including mast cells, eosinophils, neutrophils, lymphocytes, and macrophages) in the airway epithelium decreased from a pretreatment mean of 873 ± 139 cells per square millimeter to

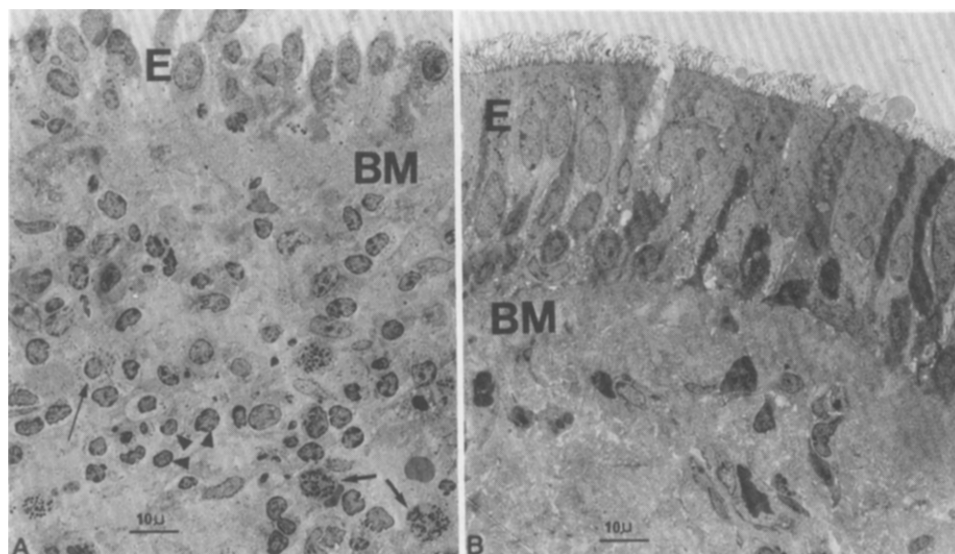


Fig. 3. A, Electron microscopic picture of a section of a biopsy specimen obtained from airway of a patient with extrinsic asthma of 9-month duration. Patient has a highly damaged airway epithelium (*E*). Deeper in lamina propria, beneath basement membrane (*BM*), an intense inflammatory reaction can be observed. Different types of inflammatory cells, eosinophils (*thick black arrows*), lymphocytes (*arrow heads*), and plasma cells reflect chronic inflammation. Mast cells (*thin black arrow*) are highly degranulated; bar, 10 μ m. (Original magnification $\times 1300$.) **B,** Airway of same patient after 3 months of inhaled budesonide treatment. Normal airway epithelium (*E*) with ciliated and goblet cells is restored on basement membrane (*BM*), and inflammatory cells have disappeared. Picture is comparable to that obtained in normal airways (Original magnification $\times 1300$.)

339 ± 33 cells per square millimeter ($p < 0.01$). In the terbutaline-treated group, the total number of cells did not decrease significantly, ranging from 792 ± 88 to 617 ± 100 cells per square millimeter ($p = 0.8$). Thus, the difference between the changes in the two groups was significant ($p < 0.01$). The number of cells (including mast cells, eosinophils, neutrophils, lymphocytes, plasma cells, macrophages, and monocytes) in the lamina propria of specimens from the budesonide-treated group decreased from a mean of 1471 ± 329 to 493 ± 73 cells per square millimeter ($p < 0.01$) (Fig. 3 *A* and *B*). The total number of cells in the lamina propria from the terbutaline-treated group also decreased significantly, from 1021 ± 96 to 583 ± 149 cells per square millimeter ($p < 0.05$). The difference between the two groups in the total number of cells in the lamina propria, therefore, was not significant.

With respect to individual cell types, several significant changes were found. Inhaled budesonide decreased the number of eosinophils in the airway epithelium ($p < 0.05$) (Fig. 4, *A*), and the decrease was significantly more in the budesonide-treated group ($p < 0.001$). Furthermore, eosinophils were found in the epithelium of only one budesonide-treated patient

and in the lamina propria of only two of the seven patients in this group (Fig. 5, *A*). In contrast, eosinophils were observed in the epithelium and in the lamina propria of four terbutaline-treated patients (Fig. 5, *B*). The number of lymphocytes in the epithelium (Fig. 4, *A* and *B*) and lamina propria (Fig. 6, *A* and *B*) was reduced significantly ($p < 0.05$) in biopsy specimens from both treatment groups (budesonide in Figs. 4, *A*, and 6, *A*, and terbutaline in Figs. 4, *B* and 6, *B*). In addition, both treatments reduced the number of mast cells and plasma cells in the lamina propria ($p < 0.05$).

DISCUSSION

This study demonstrated that treatment with inhaled budesonide for 3 months improves the clinical status of patients with asthma with a short history of the disease. There are several indicators of improvement: a decrease in asthma symptom scores, significant increases in FEV₁ and FVC, and in morning and evening PEFR values. When we compared the effect of treatment on bronchial responsiveness in the two groups, the decrease was very significant after 6 weeks of budesonide treatment and almost reached significance after 12 weeks of treatment. These results agree with

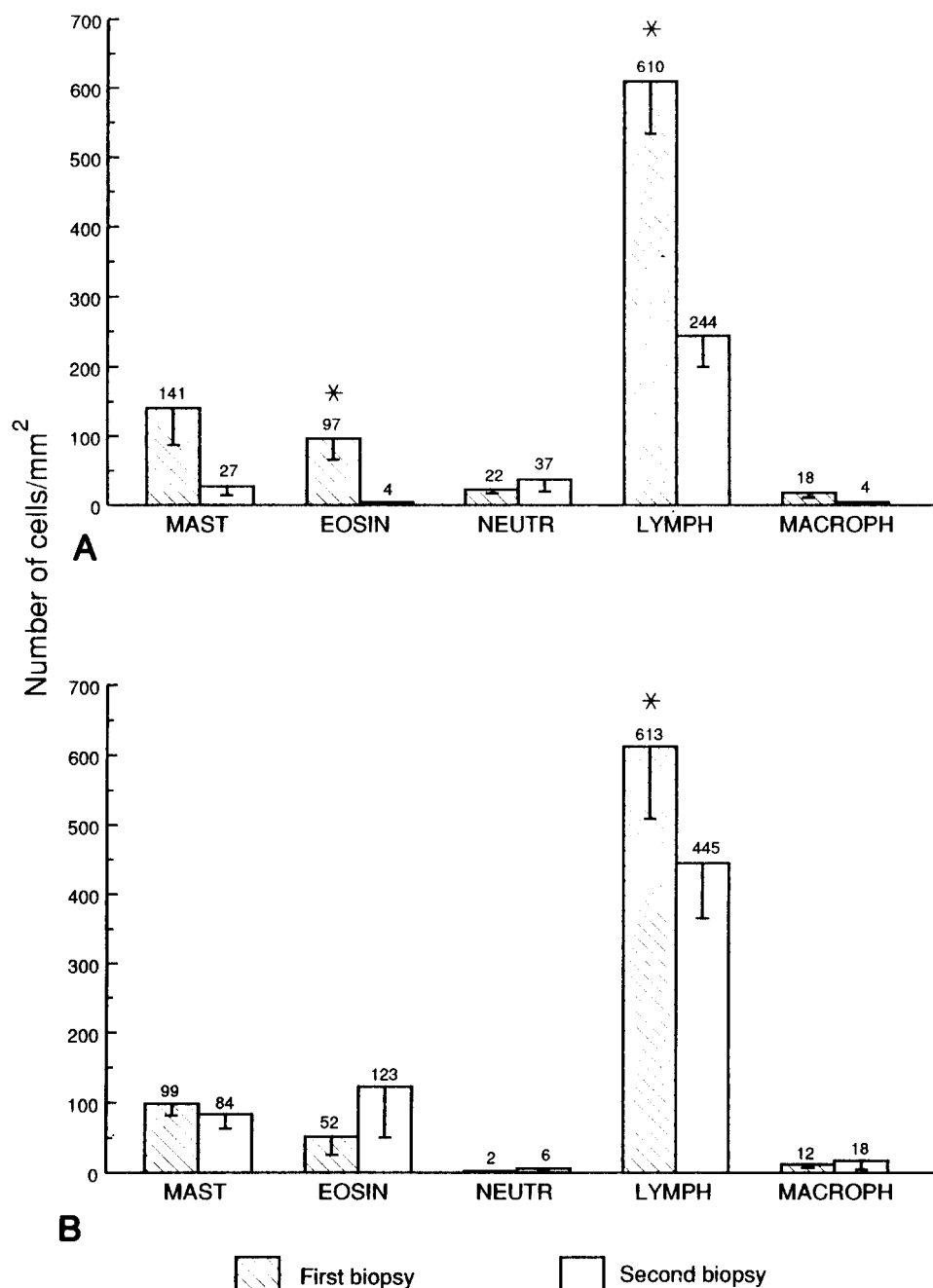


Fig. 4. **A**, Budesonide treatment decreased significantly ($*p < 0.05$) the number of eosinophils (*eosin*) and lymphocytes (*lymph*). **B**, Terbutaline treatment decreased significantly only lymphocytes in the bronchial epithelium. Neither therapies had any significant effect on number of epithelial mast cells (*mast*), neutrophils (*neutr*), or macrophages (*macroph*). First biopsy specimens were obtained just before randomization. Second biopsy specimens were obtained after 3 months of treatment. Mean $\pm$ SE values are presented.

results obtained with a larger number of similar patients in a previously reported study.⁸ The beneficial effect of inhaled corticosteroid therapy administered during different periods of time has been demonstrated in a number of other studies.^{8, 10, 19, 20}

In the present study, changes in symptoms and in

pulmonary function have been associated with information concerning mucosal structure and inflammatory cells obtained from biopsy specimens of the airways. Besides improving the clinical status and bronchial hyperresponsiveness, the number of eosinophils in the airway epithelium of every budesonide-treated

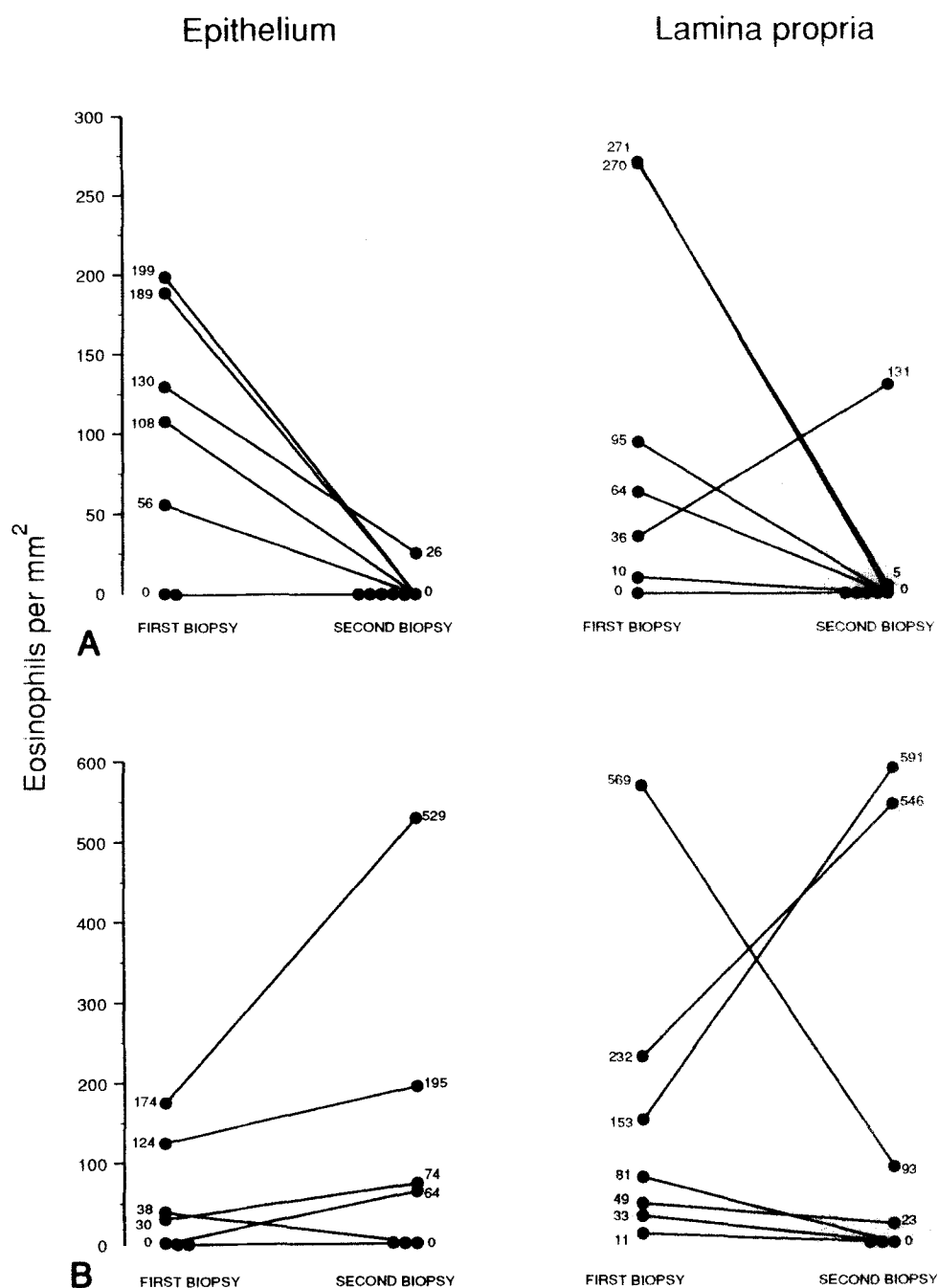


Fig. 5. **A**, Effect of budesonide and **B**, terbutaline treatment on both epithelial and lamina propria eosinophils in airways of each individual asthma patient. First biopsy specimens were obtained just before randomization. Second biopsy specimens were obtained after 3 months of treatment.

patient was decreased. This change occurred in both allergic and nonallergic patients, even though the second biopsy specimens were obtained during the pollen season in Finland, that is, between April 12 and Aug. 11. Conversely, the number of eosinophils increased in four patients receiving the β_2 -agonist treatment. Thus, there was a very significant difference in the effect on airway eosinophils between the inhaled cor-

ticosteroid and the β_2 -agonist. This effect may be important at the onset of the disease as well as later in its course, for example, during exacerbations of asthma, when more effective anti-inflammatory therapy is recommended.¹⁴

The present study also demonstrated that the inhaled corticosteroid diminished the overall inflammatory reaction in the airways. In addition to the disappearance

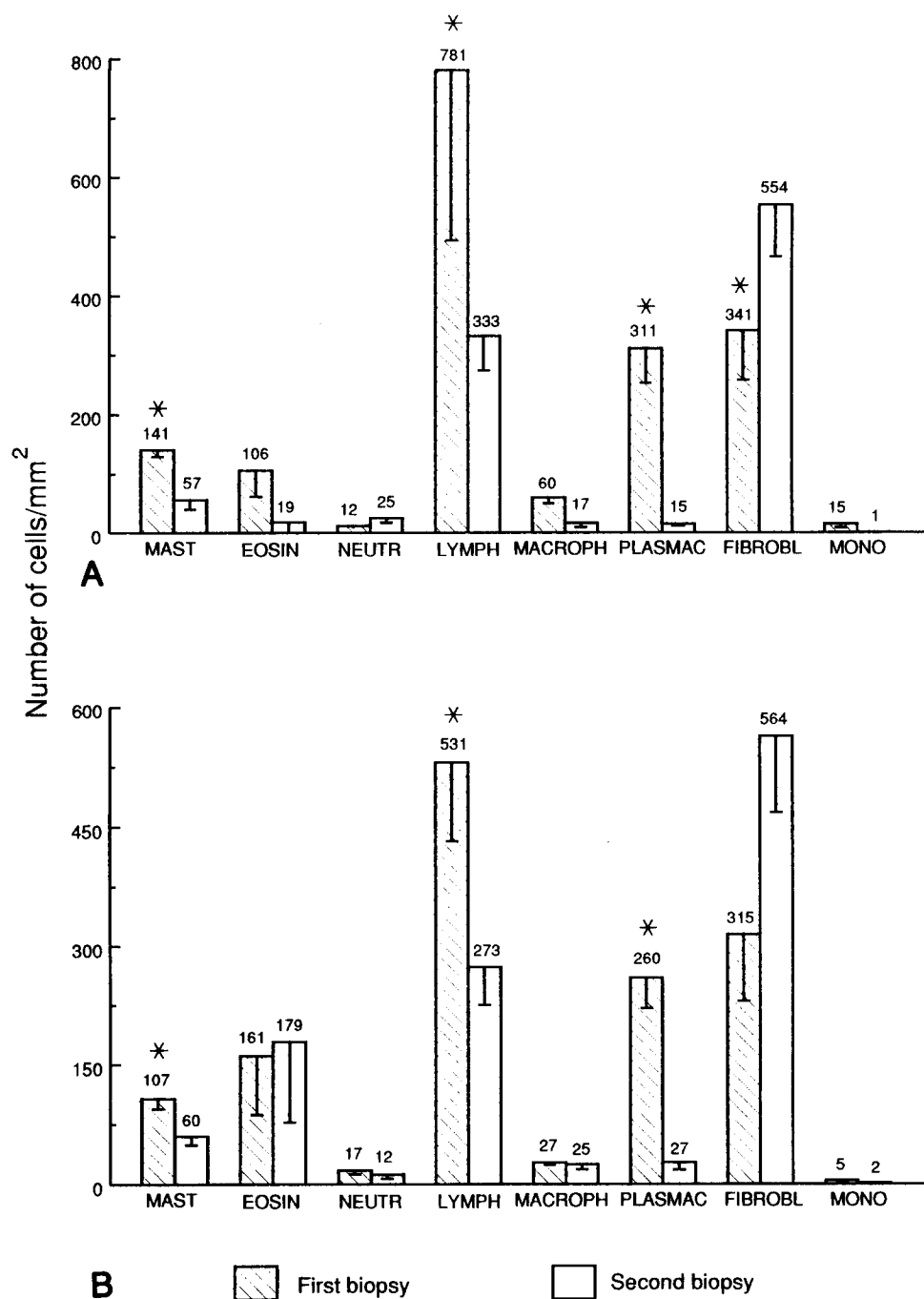


Fig. 6. In bronchial lamina propria, both **A**, budesonide and **B**, terbutaline treatments decreased significantly ($*p < 0.05$) number of mast cells (*mast*), lymphocytes (*lymph*), and plasma cells (*plasmac*). Budesonide treatment increased the number of fibroblasts (*fibrobl*). Neither therapies had any effect on eosinophils (*eosin*), neutrophils (*neutr*), macrophages (*macroph*), and monocytes (*mono*) in the bronchial lamina propria. First biopsy specimens were obtained just before randomization. Second biopsy specimens were obtained after 3 months of treatment. Mean $\pm$ SE values are presented.

of eosinophils, the number of mononuclear cells decreased greatly. This change was accompanied by an improvement in the structure of the epithelium; a pseudostratified ciliated epithelium with intraepithelial

nerves was restored. These observations suggest that the steroid not only affected the number of different inflammatory cells but also that it reversed a destructive process in the epithelium, a process that involves

a change from a ciliated epithelium to goblet cell hyperplasia, metaplasia, and possibly, epithelial shedding. Based on studies in animals, goblet cell hyperplasia is considered to be a common reaction of epithelium to many kinds of irritation, for example, mechanical and radiation injury, irritant gases, infectious agents, and carcinogens. The loss of the vulnerable ciliated cells is held to be one of the first signs of epithelial destruction.²¹ For example, in children with a chronic cough after an earlier lower respiratory tract infection, the number of ciliated cells is inversely related to inflammatory events in the epithelium.²² In contrast, the ratio of ciliated cells to goblet cells in normal human airways has been reported as varying between three and 10,² which is similar to the c/g index observed after inhaled corticosteroid treatment in the present study.

Inhibition of the production of cytokines by mononuclear cells has been postulated as a mechanism of action of corticosteroids. Cytokines are potent proinflammatory mediators that prime granulocytes for an enhanced generation of leukotrienes.²³ Leukotrienes may intensify the inflammatory reaction partly as a result of their being chemotactic for granulocytes and partly by increasing vascular permeability.

For blinding purposes, a relatively moderate dose of terbutaline was used in the present study to avoid tremor and palpitations. Even so, clinical symptoms and some lung functions for example, FVC, improved with this treatment. In this study, as in our earlier study,⁸ this moderate dose of terbutaline did not increase bronchial responsiveness, whereas with higher doses of β_2 -agonists, this may occur.^{19, 20, 24} Long-term treatment with terbutaline decreased the number of lymphocytes in the epithelium and the number of lymphocytes, mast cells, and plasma cells in the lamina propria of the airways, but it did not decrease the number of eosinophils. These effects of β_2 -agonists on inflammatory cells may be related to their ability to reduce the number of lymphocytes, but it could also be related to their ability to reduce the exudation of plasma.²⁵ Since no placebo-treated group was included in the present study, we cannot exclude a placebo effect as well. In addition, since the two patients who received a 6-day course of oral prednisolone during the period of active treatment were in the terbutaline-treated group, we cannot exclude the possible anti-inflammatory effect of treatment with this corticosteroid on the combined results of the group as a whole.

Until the present time, the effect of therapies administered to patients with asthma has been judged by clinical symptoms and pulmonary function measurements. However, with information obtained from examination of bronchial biopsy specimens from pa-

tients with asthma, we know that even clinically mild and newly diagnosed asthma is associated with an inflammatory reaction in the airways. Effects on inflammation produced by treatment may not be fully recognized by pulmonary function measurements and bronchial-challenge testing. With bronchial biopsy specimens we have been able to demonstrate that inhaled budesonide, in comparison with terbutaline, not only improves lung function and bronchial hyperresponsiveness in patients with newly diagnosed asthma but also improves the structure of their bronchial epithelium and reduces the number of eosinophils in their bronchial walls.

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Mapping human T cell epitopes on phospholipase A₂: The major bee-venom allergen

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*Phospholipase A₂ (PLA₂), the major bee-venom allergen, was purified by gel filtration, inactivated by denaturing, and carboxymethylating its cysteine residues. Peripheral blood mononuclear cells from an individual (HLA-DR2 [15], Dw52, DQ1 and DQ3) allergic to bee stings were used to generate cell lines specific for PLA₂ and a control antigen, tetanus toxoid. These lines were 90% CD3⁺, 64% CD4⁺ and 20% CD8⁺ by fluorocytometry analysis. T-lymphocyte epitope mapping done with 12 overlapping synthetic peptides of PLA₂ revealed two immunodominant epitopes. These epitopes correspond to amino acid sequences 50 to 69 and 83 to 97 of PLA₂. Cytokine interleukin-4 and Interferon- γ secretion was studied from PLA₂- and tetanus toxoid-specific cell lines. Interleukin-4 secretion was common to both cell lines but only tetanus-toxoid cell lines secreted interferon- γ . No interferon- γ was found to be secreted by PLA₂-specific cell line in response to stimulation by PLA₂ or the two immunodominant peptides. (*J ALLERGY CLIN IMMUNOL* 1992;90:42-51.)*

Key words: T-lymphocyte, cytokine, T cell epitopes, allergen, phospholipase A₂

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Bee-sting anaphylaxis is a life-threatening disorder mediated by bee venom-specific IgE antibodies.¹ Although bee venom consists of various constituents, including acid P, melittin, apamin, hyaluronidase, PLA₂, and peptide 401, PLA₂ is its major allergen. More than 90% of patients with bee-venom anaphylaxis have an IgE-antibody response to PLA₂.² IgE-antibody synthesis is closely regulated by T-lymphocytes.³⁻⁷ For an IgE response to occur, the T-lymphocyte receptor must recognize a peptide of the allergen