

Inhaled beclomethasone dipropionate downregulates CD4 and CD8 T-lymphocyte activation in peripheral blood of patients with asthma

Maria Majori, MD,^a Maria Laura Piccoli, BS,^a Stefano Bertacco, MD,^a
Angelo Cuomo, MD,^a Luca Cantini, MD,^b and Alberto Pesci, MD^a Parma, Italy

Background: Previous studies demonstrated a downregulation of T-lymphocyte (CD3⁺ cells) activation in peripheral blood after treatment with inhaled corticosteroids in patients with asthma.

Objective: This study was carried out to evaluate the effect of inhaled corticosteroids on CD4 and CD8 T-lymphocyte activation, respectively.

Methods: We examined the expression of three surface activation markers (CD25, HLA-DR, and very late activation antigen 1) on circulating CD4⁺ and CD8⁺ T-cell subsets in subjects with asthma ($n = 23$) before and 8 weeks after treatment with inhaled beclomethasone dipropionate dry powder (daily dose, 800 μ g).

Results: Beclomethasone dipropionate treatment had a marked effect in reducing the expression of the activation marker CD25 ($p < 0.01$) in both CD4⁺ and CD8⁺ T-cell subsets in peripheral blood of patients with asthma. However, no correlation was found between the downregulation of CD4 and CD8 T-lymphocyte activation and the improvement in physiologic indices of disease activity.

Conclusions: These data add to the view that CD4⁺ and CD8⁺ T lymphocytes in peripheral blood of patients with asthma are in an activated state that is downregulated by inhaled corticosteroids. (*J Allergy Clin Immunol* 1997;100:379-82.)

Key words: Asthma, beclomethasone dipropionate, inhaled corticosteroids, peripheral blood lymphocytes

Inhaled corticosteroids have been shown to be the most effective therapy available in the management of bronchial asthma.¹ One of the major targets of corticosteroids is the T cell, which is thought to play a key role in the pathogenesis of asthma.² Activated T cells produce lymphokines such as IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor, which mediate the mobilization and activation of granulocytes. Of these, eosinophils are particularly implicated in causing the bronchial mucosa damage that is thought to underlie the clinical manifestations of asthma.³

Abbreviations used

BDP:	Beclomethasone dipropionate
mAb:	Monoclonal antibody
PEF:	Peak expiratory flow
VLA-1:	Very late activation antigen 1

Although T-cell attraction and activation in the bronchial compartment is most evident in asthma, signs of activation of T cells in the peripheral blood can also be measured. In acute severe asthma, T-lymphocyte activation has been shown to be related to clinical expression of the disease as reflected by the increase of activated CD4⁺ T lymphocytes in peripheral blood, and the ability to reduce this with oral or parenteral administration of corticosteroids parallels with clinical improvement.⁴ Recent studies^{5,6} have shown that inhaled corticosteroids could also downregulate T-lymphocyte activation in peripheral blood of patients with asthma.

The aim of this study was to evaluate this effect on CD4⁺ and CD8⁺ T-cell subsets, respectively. For this purpose we examined the effect of 8 weeks of inhaled beclomethasone dipropionate (BDP) dry powder on the expression of three surface activation markers—the IL-2 receptor (CD25), the human leukocyte antigen HLA-DR, and the very late activation antigen 1 (VLA-1)—on circulating CD4⁺ and CD8⁺ T-cell subsets in patients with chronic asthma.

METHODS

Subjects

A group of 23 patients with asthma from the outpatient department of Parma Rasori Hospital (13 men and 10 women; mean age, 41.1 ± 14.2 years; range, 20 to 63 years), who fulfilled current criteria for corticosteroid therapy (symptoms inadequately controlled by inhaled β_2 -agonist therapy),^{7,8} were invited to participate in the study. All subjects had a clinical diagnosis of chronic asthma (≥ 1 year in duration) treated by inhaled β_2 -agonist alone. They all had FEV₁ equal to or greater than 60% and less than or equal to 90% of predicted value and a positive response to the reversibility test, defined as an increase of at least 15% in FEV₁ 30 minutes after 2 puffs of salbutamol (2×100 μ g) administered through metered-dose inhalers. Diurnal variability of peak expiratory flow (PEF), monitored over a 2-week run-in period, was not greater than 20%. Patients requiring theophylline, anticholinergics, oral

From ^aIstituto di Clinica delle Malattie dell'Apparato Respiratorio, dell'Università and ^bDirezione Medica Chiesi Farmaceutici, Parma.

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Reprint requests: Alberto Pesci, MD, Istituto di Clinica delle Malattie Apparato Respiratorio, Via Rasori no. 10, 43100 Parma, Italy.

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TABLE I. Lymphocyte subpopulations and percentages of peripheral blood CD4⁺ and CD8⁺ cells expressing the activation markers HLA-DR and VLA-1 from patients with asthma before and after treatment with inhaled BDP dry powder

	CD3	CD19	CD23	CD4	CD8	CD4/HLA-DR	CD4/VLA-1	CD8/HLA-DR	CD8/VLA-1
Before									
Median	71.8	10.4	6.3	43.6	30.8	2.4	0.4	2.9	0.2
Range	56.9-83.2	4.9-18.1	2.6-13.6	30.6-55.3	17.4-51.4	0.9-14.5	0-3.4	0.7-18.9	0-1
After									
Median	71.4	12.3	8.4	46.8	26.9	2.4	0.5	2.4	0.1
Range	63.4-85	4-21.5	1-13.5	29-58.5	18.8-49.9	1-12.6	0-3.1	0.7-11.8	0-1.1
<i>p</i> Value	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, Not significant.

corticosteroids, long-acting inhaled bronchodilators, oral bronchodilators, or antihistamines were excluded from the study. Respiratory symptoms were controlled with inhaled short-acting bronchodilators (salbutamol) on a continuous basis or on demand. Thirteen patients were classified as atopic; presence of atopy was not a prerequisite for selection. Atopy was assessed by skin prick tests with a standard battery of eight common inhalant allergens (*Dermatophagoides pteronyssinus*, *D. farinae*, mixed grass pollen, cat allergen, dog dander, birch pollen, feathers, and *Aspergillus fumigatus*).

Each patient gave informed, written consent to participate in the study. The protocol was approved by the Ethics Committee of University of Parma.

Study protocol

After selection, all subjects were studied for a preliminary 2-week run-in period, during which they recorded their PEF as the best of three pretreatment measurements taken twice daily with the use of a mini-Wright portable peak flow meter (Markos, Monta, Italy). On day 14, FEV₁ and PEF were measured by a dry spirometer (Spiroflow; Morgan, Kent, U.K.), and a peripheral venous blood sample was taken for lymphocyte subset analyses and measurements of plasma cortisol levels. Subjects then initiated treatment with BDP dry powder administered through the Pulvinal multidose inhaler (Chiesi Farmaceutici SpA, Parma, Italy), 400 µg, twice daily for 8 weeks. On completion of the 8-week treatment period, pulmonary function and blood sample measurements were repeated. The study was conducted outside the pollen season.

Analysis of lymphocytes

Specific binding of monoclonal antibodies (mAbs) was analyzed by direct immunofluorescence according to standard methods recommended by Becton-Dickinson Monoclonal Center (Mountain View, Calif.) by using a flow cytometer (FACScan, Becton Dickinson). Briefly, 100 µl of heparinized whole blood was incubated in the presence of saturating concentrations of fluorescein- or phycoerythrin-conjugated mAbs at room temperature for 20 minutes. Erythrocytes were lysed by adding 2 ml of lysing solution for 10 minutes. Cells were washed twice with phosphate-buffered saline containing 2% fetal calf serum and 0.1% sodium azide. Cytofluorometric analysis was performed by imposing an electronic gate on forward-scatter and side-scatter dot plots for peripheral blood lymphocytes. The number of immunofluorescence-positive cells was determined in 10,000 analyzed cells. Specific binding of mAbs was controlled by subtraction of isotype-matched mouse immunoglobulins. Monoclonal antibodies against CD3 (T cells), CD4 (T helper-inducer cells), CD8 (T suppressor-cyto-

toxic cells), CD19 (B cells), CD25 (p55 IL-2 receptor), HLA-DR, and CD23 (B cells expressing the low-affinity Fc IgE receptor) were purchased from Becton Dickinson; and mAb to VLA-1 was obtained from T Cell Science (Cambridge, U.K.).

Simultaneous T-cell subset analysis (CD4 and CD8) and activation study (expression of activation markers CD25, HLA-DR, and VLA-1) were performed on peripheral blood lymphocytes.

Plasma cortisol levels

Blood samples were drawn from antecubital veins between 8:00 and 9:00 am to avoid spontaneous circadian variations of cortisol plasma levels. A fraction of the blood sample was centrifuged at 800 rpm for 20 minutes and then transferred to test tubes of 0.5 ml. Test tubes were immediately frozen at -80°C pending analysis. All samples were analyzed, after natural defrosting with a Cortisol TDx/TDxFLx kit (Abbott, Roma, Italia), by using an immunologic dosage with fluorescence to polarized light (FPIA) technique.

Data analyses

Results were expressed as medians and range or means ± SD when appropriate. For statistical analysis, Bartlett's test for homogeneity of variances was conducted. If variances were homogeneous, the parametric statistic (Student's *t* test for paired data) was used; if not, the nonparametric test (Wilcoxon's signed-rank test) was used. Correlations were examined by Spearman's rank correlation coefficient. A *p* value less than 0.05 was considered significant.

RESULTS

All the patients completed the scheduled treatment period, and no adverse reactions were reported. The treatment resulted in a significant overall improvement in lung functional parameters, such as FEV₁ (2.3 ± 0.7 L to 2.9 ± 0.8 L, *p* < 0.05) and PEF (4.3 ± 1.5 L/sec to 5.6 ± 1.6 L/sec, *p* < 0.05). Plasma cortisol levels did not show any statistically significant modifications after the treatment period (15.7 ± 13.9 µg/ml to 15.3 ± 10.4 µg/ml, not significant).

Flow cytometric analysis of peripheral blood lymphocytes before and 8 weeks after treatment with inhaled BDP dry powder showed no significant differences in the percentage of T lymphocytes (CD3⁺), B lymphocytes (CD19⁺), and CD23⁺ cells (Table I). There were no changes in either the CD4⁺ or CD8⁺ T-cell subsets, nor

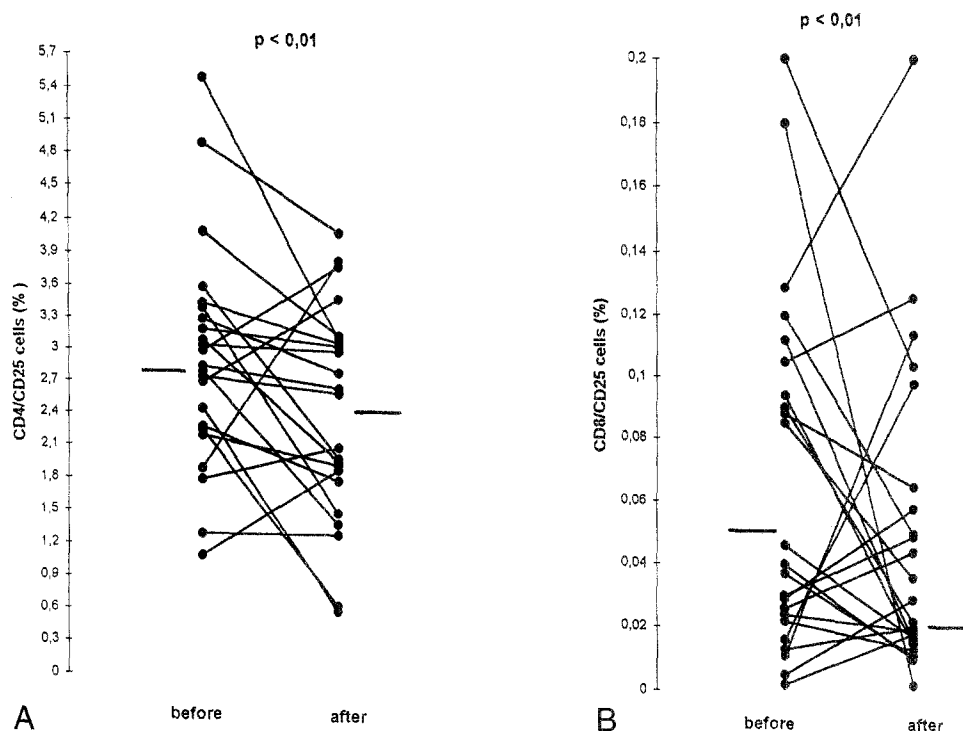


FIG. 1. Percentages of peripheral blood CD4⁺ cells (A) and CD8⁺ (B) cells expressing the activation marker CD25 from patients with asthma before and after treatment with inhaled BDP dry powder. Horizontal bars represent group mean values.

was there any change in the expression of HLA-DR and VLA-1 in either CD4⁺ or CD8⁺ cells (Table I). However, comparison of the CD25 activation marker did show significant reduction after treatment both in CD4⁺ ($p < 0.01$) and CD8⁺ ($p < 0.01$) T-cell subsets (Fig. 1). Changes in the expression of CD25 on CD4⁺ and CD8⁺ cells did not correlate with the improvement in physiologic indices.

No differences were observed between data from patients with atopic asthma and those with nonatopic asthma.

DISCUSSION

This study shows a downregulation of CD4 and CD8 T-lymphocyte activation in peripheral blood in parallel with an improvement in lung function parameters after treatment with inhaled BDP dry powder in patients with chronic asthma. Our study confirms and extends previous observations that demonstrated a downregulation of T-cell activation in peripheral blood after treatment with inhaled corticosteroids, such as BDP⁵ and budesonide,⁶ in patients with asthma. These studies examined T-cell activation only on CD3⁺ cells, whereas we demonstrated a downregulation of T-cell activation in both CD4⁺ and CD8⁺ T-cell subsets.

Increased T-cell activation in peripheral blood of patients with asthma may reflect either a "spillover" of T cells activated from the bronchial mucosa or an influence of the bronchial microenvironment on circulating T lymphocytes.⁹ Indeed, analysis of peripheral blood and

bronchoalveolar lavage lymphocytes from patients with atopic asthma after allergen bronchial challenge has demonstrated dynamic exchanges of T lymphocytes between these two compartments.¹⁰ Thus the downregulation of CD4 and CD8 T-lymphocyte activation, which we observed in peripheral blood after BDP treatment, may reflect a reduction of the inflammatory process in the bronchial mucosa. The alternative explanation of a slight "systemic downregulating effect" of inhaled corticosteroids on T-lymphocyte activation in peripheral blood seems less likely because no clinically relevant adverse effects on host defense mechanisms were reported¹ and because the plasma cortisol levels of our patients were unchanged after treatment.

The decreased numbers of CD25-expressing but normal numbers of HLA-DR- and VLA-1-expressing CD4⁺ and CD8⁺ cells after therapy may be explained by a better specificity for T-cell activity of this marker. Indeed, CD25 is the receptor for the T-cell autocrine growth factor IL-2, whereas the physiologic function of HLA-DR and VLA-1, if any, is unclear.

The downregulation of CD8, other than CD4, T-lymphocyte activation may be important in view of the now increasing evidence for the existence of T_{H2}-like CD8⁺ T cells.¹¹⁻¹⁵ In fact, it is now clear that CD8⁺ cells may also secrete cytokines such as IL-4 and IL-5, which are essential cofactors in IgE synthesis and the accumulation and activation of eosinophils, respectively.^{16, 17} In a previous study Walker et al.¹⁸ demonstrated an up-regulation of CD8 T-lymphocyte activation in bron-

choalveolar lavage and peripheral blood of patients with nonallergic asthma. As far as we know, our study is the first to demonstrate a downregulation of CD8 T-lymphocyte activation in peripheral blood of patients with asthma after treatment with inhaled corticosteroids. We found no difference between atopic and nonatopic patients.

In acute severe⁴ and moderate to severe¹⁹ asthma, T-lymphocyte activation in peripheral blood has been shown to be related to clinical expression of the disease as reflected by the increase of CD4⁺ T lymphocytes activated in peripheral blood and the ability to reduce this increase by means of parenteral or oral administration of corticosteroids in parallel with clinical improvement. However, we failed to show any positive correlation between clinical improvement and decrease in T-cell activity in peripheral blood. This discrepancy may be explained by the fact that our patients had less severe disease. Indeed, the importance of T cells in the pathogenesis of bronchial asthma may be dependent on the severity of the disease.²⁰ Another possible explanation for the lack of correlation between clinical improvement and decrease in T-cell activity in peripheral blood is that because asthma is a multifactorial disease, symptoms may be triggered by several nonimmunologic factors that determine disease activity (e.g., viral infections), rendering the demonstration of clear correlations between cells, their activation, and disease activity very difficult.

In conclusion, our study shows a downregulation of peripheral lymphocyte activation in both CD4⁺ and CD8⁺ T-cell subsets in parallel with an improvement in lung function parameters after treatment with inhaled BDP dry powder in patients with chronic asthma. These data add to view that CD4⁺ and CD8⁺ T lymphocytes in peripheral blood of patients with asthma are in an activated state that is susceptible to downregulation by inhaled corticosteroids.

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A new murine model of pulmonary eosinophilic hypersensitivity: Contribution to experimental asthma

Ana Lúcia Pereira de Siqueira, BSc, Momtchilo Russo, MD, PhD,
Ana Angélica Steil, BSc, Sandra Facincone, BSc, Mario Mariano, DVM, PhD, and
Sonia Jancar, BSc, PhD São Paulo, Brazil

Background: We have recently described a model of hypersensitivity reaction in the mouse paw, which induces a typical late-phase reaction with a marked eosinophilic infiltrate.

Objective: In the search for a murine model of asthma, this model was adapted to the lungs and compared with other models of pulmonary hypersensitivity.

Methods: A fragment of heat-coagulated hen's egg white was implanted subcutaneously, and 14 days later, the mice were challenged intratracheally with aggregated ovalbumin. Comparison was made with a group that received subcutaneous injection of soluble ovalbumin in alumen, challenged as described above and with four additional protocols of immunization and challenge.

Results: Forty-eight hours after challenge, the percentage of eosinophils was higher in the egg white implant group (35%) than in the group immunized with ovalbumin in alumen (10.4%). The eosinophil peroxidase activity in lung homogenates of the first group was also significantly higher (529 ng/ml) than that of the second group (43 ng/ml). These results were reproduced in five different mouse strains. Compared with five different models of lung hypersensitivity, the egg white implant model was unique in terms of persistence of the pulmonary eosinophilia. Histopathologic analysis of the lungs of mice immunized with egg white implant showed peribronchial, perivascular, and intraepithelial eosinophil infiltration; morphologic characteristics of bronchoconstriction; and patchy epithelial shedding. At 21 days, in addition to persistence of eosinophil infiltrate, enlarged alveoli, reflecting air trapping, were observed.

Conclusion: On the basis of the characteristics of the model described here, we propose it as a suitable murine model of asthma. (*J Allergy Clin Immunol* 1997;100:383-8.)

Key words: Asthma, late-phase reaction, eosinophil, mice, eosinophil peroxidase

Abbreviations used

BAL:	Bronchoalveolar lavage
EPO:	Eosinophil peroxidase
EWI:	Egg white implant
LPR:	Late-phase reaction
OA-AL:	Ovalbumin in alumen
PBS:	Phosphate-buffered saline

Asthma is a chronic inflammatory disease of the airways. It is characterized by intermittent, reversible airway obstruction and by bronchial hyperreactivity. It appears that the airway inflammatory reaction is mediated by T-helper type 2 lymphocytes (T_{H2}), eosinophils, and mast cells.¹⁻⁴ Lung eosinophilia correlates with T-lymphocyte infiltration.^{5,6} A large number of eosinophils is found during the late-phase reaction (LPR) in the lungs.⁷ Blocking the T_{H2} development by IL-12 administration in vivo inhibits both the allergen-induced eosinophilia and bronchial hyperreactivity.⁶ The pattern of cytokines produced by T_{H2} cells promotes proliferation of mast cells, stimulates IgE and IgG₁ production, and facilitates eosinophil migration, activation, and survival.^{7,8} Indeed, it was shown that airway eosinophilia and hyperreactivity did not occur in IL-5-deficient mice.⁹ Furthermore, the severity of bronchial hyperreactivity, which is one of the major characteristics of asthma, parallels the intensity of pulmonary eosinophil infiltration.¹⁰⁻¹² However, Pretolani et al.¹³ showed that the sole presence of eosinophils in the airways is not sufficient to induce bronchial hyperreactivity. They suggested that eosinophil activation correlates better with hyperreactivity than eosinophil number per se. Indeed, products of activated eosinophils, such as cytotoxic proteins, were shown to damage the epithelium of the airways. It was shown that injection of eosinophil-derived cationic proteins into the airways causes desquamation of the epithelium and bronchial hyperreactivity.¹⁴⁻¹⁷ These cells can also produce leukotrienes and platelet activating factor.^{18,19} Leukotrienes induce bronchoconstriction and mucus secretion; platelet activating factor increases eosinophil infiltration into the airways.^{20,21}

The advances in the understanding of the mechanisms involved in the pathogenesis of asthma came from studies with animal models. A great variety of experi-

From the Department of Immunology, Institute of Biomedical Sciences, University of São Paulo. Sandra Facincone and Ana Lúcia Pereira de Siqueira are postgraduate students from the Department of Pathology, School of Veterinary Medicine and the Department of Immunology, respectively, of the University of São Paulo.

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Reprint requests: Sonia Jancar, BSc, PhD, Departamento de Imunologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, Av. Prof. Lineu Prestes 2415, 05508-900, São Paulo, Brazil.

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