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Cell surface antigen expression by peripheral blood monocytes in allergic asthma: results of 2.5 years therapy with inhaled beclomethasone dipropionate

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AT present, inhaled glucocorticoids are widely accepted as the therapy of choice in chronic asthma. Treatment with inhaled glucocorticoids significantly suppresses local airway inflammation in asthmatics, but may also have systemic effects, e.g. a reduction of the number of circulating hypodense eosinophils or a down-modulation of HLA-DR antigen (Ag) expression by T lymphocytes in peripheral blood. However, the effect of long-term therapy with inhaled glucocorticoids on peripheral blood monocytes (PBM), which are the precursors of the most numerous cell type in the lung, the alveolar macrophage, have not yet been evaluated. We therefore investigated the expression of various cell surface Ag on PBM from non-smoking patients with allergic asthma who were treated for 2.5 years with a β_2-receptor agonist plus either an inhaled glucocorticoid (beclomethasone dipropionate, BDP) ($n = 4$) or an anticholinergic or placebo ($n = 8$). We compared the results with healthy volunteers ($n = 7$). Long-term treatment of allergic asthmatics with inhaled BDP, but not anticholinergic or placebo therapy, was associated with a significantly lower CD11b Ag expression ($p < 0.04$) and higher expression of CD13, CD14 and CD18 Ag ($p < 0.05$, $p < 0.02$ and $p < 0.04$, respectively) when compared with the healthy control subjects ($n = 7$). Most interestingly, PBM of asthmatics treated with inhaled BDP expressed an almost two-fold higher level of CD14 Ag on their cell surface than PBM of patients treated with anticholinergic or placebo ($p < 0.03$). No significant differences in the expression of CD16, CD23, CD25, CD32 and CD64 Ag or HLA-DR were observed between PBM from the different patient groups or healthy controls. Taken together, this study shows that long-term local therapy with inhaled BDP coincides with an altered expression of at least one cell surface Ag on PBM from allergic asthmatics.

Key words: Allergic asthma, Inhaled glucocorticoids, Peripheral blood monocytes

Introduction

One of the major histopathological findings in asthma is chronic inflammation of the airways. In bronchial mucosal biopsies, this inflammation is characterized by an accumulation of mononuclear phagocytes, eosinophils, mast cells and T lymphocytes.[1–4] These inflammatory cells have also been described in the bronchoalveolar lavage fluid,[5–7] which, in addition, has been shown to contain elevated levels of various inflammatory mediators.[7,8] It is generally believed that the airway inflammation in asthma results from the concerted actions of the different types of inflammatory cells and their products, and underlies some of the clinical symptoms.[1,3,5]

The most numerous cell type in both the normal and asthmatic lung is the alveolar macrophage. This cell type is nowadays known to play a central role in initiating, perpetuating, and reducing inflammatory processes.[9–12] Monocytes and macrophages recovered from bronchoalveolar lavage fluid of asthmatics are highly activated,[13–15] manifested in the release of a large variety of mediators which in turn modulate the inflammatory responses of eosinophils, mast cells and T cells.[7,16] Furthermore, it has been reported that alveolar macrophages of asthmatic patients express elevated levels of

low affinity receptors for IgE (CD23),[17] which are capable of inducing cellular activation in response to specific allergen.

At present, glucocorticoids are the most effective therapy for controlling airway inflammation and clinical symptoms in chronic asthma.[18,19] The precise mechanisms by which glucocorticoids reduce airway inflammation are not yet fully understood, but it is known that they modulate gene expression by binding to specific glucocorticoid-responsive elements in DNA.[20,21] One of the major mechanisms by which glucocorticoids inhibit inflammation is direct suppression of cytokine production by mononuclear phagocytes and T cells[22] or the induction of anti-inflammatory proteins such as lipocortin-1.[23] Additionally, glucocorticoids inhibit the production of pro-inflammatory mediators indirectly by suppressing the activity of several enzymes involved in the production of these mediators.

Therapy with inhaled glucocorticoids for 1–4 months results in a significant decrease in the numbers of macrophages, eosinophils, mast cells, and T cells in the bronchial epithelium and submucosa of asthmatic patients.[24–27] Only mild systemic effects have been reported with a daily dose of 1 000–2 000 μg of inhaled glucocorticoids. Numbers of peripheral blood eosinophils have been shown to be reduced after treatment with inhaled glucocorticoids.[19,28] The effect of long-term treatment with inhaled glucocorticoids on peripheral blood monocytes (PBM), the precursors of alveolar macrophages, has not yet been evaluated. Identification of the effects of therapy with inhaled glucocorticoids on peripheral blood cells may be important, as increased numbers of eosinophils and activated T cells are detected in blood of asthmatic patients, in addition to the infiltration of inflammatory cells in the airways.[29,30]

In this report, we analysed the expression of various cell surface Ag by PBM of non-smoking patients with allergic asthma who were treated for 2.5 years with an inhaled β_2-receptor agonist plus either inhaled beclomethasone dipropionate (BDP), or an anticholinergic or placebo. The patients were participants in the Dutch double-blind placebo-controlled CNSLD study.[31,32] Our data demonstrate that PBM of asthmatic patients treated with inhaled BDP, in contrast to PBM of patients treated with anticholinergic or placebo, show significant changes in the expression of CD11b, CD13, CD14 and CD18 Ag when compared with healthy control subjects. Importantly, PBM of asthmatics treated with inhaled BDP expressed significantly more CD14 Ag on their cell surface than PBM of patients who were treated with bronchodilator or placebo. These findings suggest that local inhaled glucocorticoid therapy, in addition to earlier reported effects on blood eosinophils,[19,28] may result in systemic effects on PBM.

Materials and Methods

Subjects

Twelve non-smoking allergic asthmatic patients (participating in the Dutch double-blind placebo-controlled CNSLD study)[31,32] and seven healthy volunteers were studied. The characteristics of each patient are described in Table 1. The diagnosis of asthma was made according to the criteria of the American Thoracic Society[33] and was based on a history of attacks of breathlessness and wheezing without chronic cough

Table 1. Patient characteristics

Patient number	Gender	Age (years)	Baseline FEV_1 (% of predicted)	After 2.5 years FEV_1 (% of predicted)	Baseline PC_{20} (mg/ml)	After 2.5 years PC_{20} (mg/ml)
Glucocorticoid group						
1	M	36	46.3	64.5	0.13	1.46
2	F	44	75.0	88.5	0.06	0.45
3	F	45	49.2	69.6	0.05	0.21
4	F	60	59.7	90.3	0.06	0.96
Median		44.5	54.5	79.1	0.06	0.71
Anticholinergic/placebo group						
1	F	41	54.3	53.1	0.03	0.04
2	M	42	48.7	56.5	0.24	0.87
3	M	24	61.5	54.7	0.19	0.58
4	M	44	63.5	66.3	0.02	0.05
5	M	50	65.1	62.7	0.28	0.18
6	M	57	81.6	42.3	0.14	0.04
7	F	38	57.0	43.6	0.13	0.06
8	M	26	90.0	61.5	0.13	0.02
Median		41.5	62.5	55.6	0.14	0.06

or sputum production. Chronic was defined as more than three months per year. At entry to the study, all patients showed a 20% decrease in FEV_1 resulting from inhalation of a provocative concentration of histamine of ≤ 8 mg/ml (PC_{20}).[32,34] Allergy was defined as at least two positive wheal and flare reactions to skin prick tests with twelve common aeroallergens, or a positive test to house dust mite.[32] All patients but one had a baseline reversibility $\geq 10\%$ of predicted (one patient had a reversibility of 4%). Control subjects (three male, median age 27 years; four female, median age 23 years) were healthy volunteers, taking no medication, who had no history of allergy or asthma and had negative skin prick tests. Approval for the study protocol was obtained from the Medical Ethics Committees of the participating centres. All subjects gave written informed consent.

Study design

Details of the study design have been described previously.[32] Briefly, after a double-blind randomization patients were treated with the β_2-receptor agonist terbutaline (two puffs of 250 μg q.i.d.) plus either: (A) inhaled glucocorticoid BDP, two puffs of 100 μg q.i.d., (B) anticholinergic bronchodilator (ipratropium bromide), two puffs of 20 μg q.i.d., or (C) placebo q.i.d. At the end of the study, no significant differences with regard to FEV_1 and PC_{20} were found between the groups receiving either ipratropium bromide or placebo. Therefore, the data of these groups were pooled for analysis as one single group (designated as anticholinergic/placebo group). Heparinized venous blood of patients was collected after completing 2.5 years of treatment. Heparinized blood of healthy volunteers served as control.

Isolation of peripheral blood mononuclear cells

Mononuclear cells from heparinized venous blood were isolated by Ficoll density centrifugation (Ficoll Paque; density 1.077 g/ml; Pharmacia, Uppsala, Sweden) for 15 min at room temperature (centrifugal force $1\,000 \times \boldsymbol{g}$). Peripheral blood mononuclear cells (PBMC) were washed twice at 4°C using PBS (300 mosmol; pH 7.8) supplemented with 0.5% heat-inactivated BSA (Organon Teknika; Turnhout, Belgium) and 0.05% w/v NaN_3 (PBS/BSA/NaN_3). The cell concentration was adjusted to 5×10^6 cells/ml.

Immunofluorescence staining

MoAb used in this study are listed in Table 2. CD11 and CD18 moAb were used because of the central role of leukocyte adhesion molecules in the recruitment of blood cells to inflammatory sites.[35] Cell surface aminopeptidase-N can be recognized by CD13 moAb, and may play a role in the inactivation of some

Table 2. Monoclonal antibodies used in this study

moAb	Clone (isotype)	Source
CD3	Leu-4 (IgG_1)	BD[a]
CD4	Leu-3a (IgG_1)	BD
CD8	Leu-2a (IgG_1)	BD
CD11b	44 (IgG_1)	Dr N. Hogg[b]
CD13	Q20 (IgG_{2a})	Dr C. E. van der Schoot[c]
CD14	UCHM1 (IgG_{2a})	Dr N. Hogg
CD15	VIM-D5 (IgM)	Dr W. Knapp[d]
CD16	Leu-11b (IgM)	BD
CD18	LFA-1/1 (IgG_1)	CLB, Amsterdam, the Netherlands
CD20	B1 (IgG_{2a})	Coulter Clone, Hialeah, FL
CD23	Tü1 (IgG_1)	Biotest, Dreieick, Germany
CD25	IL-2R (IgG_1)	BD
CD32	IV.3 (IgG_{2b})	Medarex Inc., West Lebanon, NH
CD64	32.2 (IgG_1)	Medarex Inc.
HLA-DR	L243 (IgG_{2a})	BD
Anti-macrophage Ag	RFD9 (IgG_1)	Dr L. W. Poulter[e]
Isotype control	(IgG_1)	BD
Isotype control	(IgG_{2a})	BD
CD1[f]	Leu-6 (IgG_{2b})	BD

[a]Becton Dickinson, Sunnyvale, CA, USA.
[b]London, UK.
[c]CLB, Amsterdam, the Netherlands.
[d]Vienna, Austria.
[e]Royal Free Hospital School of Medicine, London, UK.
[f]Used as IgG_{2b} isotype control.

inflammatory mediators.[36,37] CD14 moAb was used because it recognizes the receptor for LPS binding protein, which may determine the cellular responsiveness to bacterial products.[38] moAb recognizing Fcγ receptors (CD16, CD32, CD64) were used because these receptors play a role in the response of cells to specific antigens.[39] The presence of low affinity receptors for IgE, recognized by CD23 moAb, may lead to activation of cells in response to specific allergens.[40] Both the expression of the α-chain of the IL-2 receptor (CD25) and HLA-DR are markers of cellular activation.[27] RFD9 recognizes a cell membrane determinant present on alveolar macrophages, but virtually absent on blood monocytes, and may be used as a marker of maturation.[41] Irrelevant isotype-matched antibodies were used as controls. Reagents were diluted to optimal concentration in PBS/BSA/NaN_3. All incubations (30 min each) were carried out on ice. Each incubation was followed by two washes with PBS/BSA/NaN_3 at 4°C. For single-colour stainings, aliquots of 2.5×10^5 PBMC (in a volume of 50 µl) were first incubated with 50 µl moAb, washed, and subsequently incubated with FITC-conjugated goat anti-mouse Ig (GAM-FITC; CLB, Amsterdam, the Netherlands). After the last washing procedure, the cells were resuspended in PBS/BSA/NaN_3. For two-colour stainings, 50 µl PBMC were incubated with both a FITC-conjugated and PE-conjugated moAb, washed and eventually resuspended in FACSFLOW™ (Becton Dickinson, Sunnyvale, CA, USA).

Flow cytometry

Flow cytometric analyses were performed using a FACScan flow cytometer (Becton Dickinson) equipped with a 488 nm argon laser. Residual erythrocytes, dead cells and debris were excluded from analysis by electronic gating on the basis of forward and perpendicular light scatter. At least 7 500 events were acquired. Cell surface fluorescence was analysed after setting scatter gates on either the monocyte or lymphocyte fraction. Within the monocyte gate, cell surface fluorescence was expressed as molecules equivalent to soluble FITC (MESF). MESF values ($\times 10^4$) were obtained by interpolating cell surface fluorescence to a standard curve prepared using microspheres of known fluorescence intensities (Flow Cytometry Standards, Research Triangle Park, NC, USA). MESF values were corrected for background fluorescence by subtracting the MESF values obtained with isotype-matched control antibodies. Within the lymphocyte gate, results were expressed as the percentage of positively stained cells relative to an isotype-matched control antibody. Subpopulations of T lymphocytes were identified by two-colour labelling.

Statistical analysis

Differences between groups in either the percentage of positively stained lymphocytes or the level of cell surface fluorescence by monocytes were compared using the two-tailed Mann–Whitney *U*-test. Differences associated with p values < 0.05 were regarded as statistically significant.

Results

Long-term treatment with inhaled BDP in asthma is associated with differences in the level of cell surface Ag expression by PBM

The expression of various cell surface Ag by PBM from both non-smoking allergic asthmatics treated for 2.5 years with either inhaled BDP or anticholinergic/placebo, and healthy volunteers is summarized in Table 3. PBM from patients who received anticholinergic/placebo did not show any significant alterations in the levels of cell surface Ag expression when compared with PBM from healthy controls. In contrast, PBM from patients treated with inhaled BDP differed significantly in the level of expression of CD11b ($p < 0.04$), CD13 ($p < 0.05$), CD14 ($p < 0.02$), and CD18 ($p < 0.04$) Ag when compared with PBM from healthy controls (Fig. 1). A reduced level of CD11b Ag expression was observed, whereas the expression of CD13, CD14, and CD18 Ag was higher. The only significant difference between the two groups of asthmatic patients concerned the level of CD14 Ag expression; PBM from patients who had received inhaled BDP expressed significantly higher levels of CD14 Ag than PBM from asthmatic patients who had received anticholinergic/placebo (Table 3, Fig. 1). Both groups of patients and the control group, however, did not differ in the relative numbers of $CD14^+$ monocytes in peripheral blood (data not shown). The expression of RDF9 by PBM of asthmatics treated with inhaled BDP was significantly increased when compared with PBM of healthy controls ($p < 0.01$). However, this specific cell surface fluorescence fell within two-fold the fluorescence intensity of an isotype-matched control moAb, suggesting that monocytes express negligible levels of RFD9 Ag. No differences in the expression of CD16 (FcγRIII), CD23 (FcεRII),

Table 3. Specific cell surface fluorescence by peripheral blood monocytes

	Control group ($n=7$)	Anticholinergic/placebo group ($n=8$)	Glucocorticoid group ($n=4$)
PBMC			
% CD15$^+$	0.7 (0.5–2.0)[a]	1.8 (0.4–5.5)	1.6 (0.5–6.8)
Monocytes			
CD11b	19.2 (9.6–27.1)[a,b]	12.6 (2.1–26.4)	7.6 (4.1–13.0)[c($p<0.04$)]
CD13	5.3 (1.5–6.4)	5.4 (3.6–12.9)	8.2 (6.1–18.7)[c($p<0.05$)]
CD14	13.0 (9.4–18.7)	11.9 (7.9–19.7)	20.4 (13.7–21.5)[c($p<0.02$)d($p<0.03$)]
CD16	1.1 (0.6–2.3)	0.9 (0.6–1.6)	1.3 (0.7–1.6)
CD18	8.5 (5.7–11.0)	13.8 (6.3–33.4)	12.5 (8.8–16.1)[c($p<0.04$)]
CD23	1.0 (0.9–1.6)	1.1 (0.5–2.2)	1.3 (0.7–1.8)
CD25	0.9 (0.7–1.3)	0.9 (0.5–1.3)	1.1 (0.6–1.6)
CD32	11.7 (6.6–15.0)	13.4 (7.2–19.9)	14.0 (11.5–17.6)
CD64	7.7 (4.3–10.4)	8.4 (4.2–12.4)	7.0 (6.3–10.8)
RFD9	1.3 (0.9–1.4)	1.4 (0.6–2.4)	1.8 (1.7–2.0)[c($p<0.01$)]
HLA-DR	5.9 (3.8–6.6)	6.8 (4.8–21.4)	7.0 (5.7–9.3)

[a]Median values (range).
[b]MESF values, corrected for background fluorescence by isotype-matched control Ab, were calculated using a calibration line obtained with microspheres of known fluorescence intensities. Statistical analysis was performed using the two-tailed Mann–Whitney *U*-test.
[c]Significantly different from control group.
[d]Significantly different from anticholinergic/placebo group.

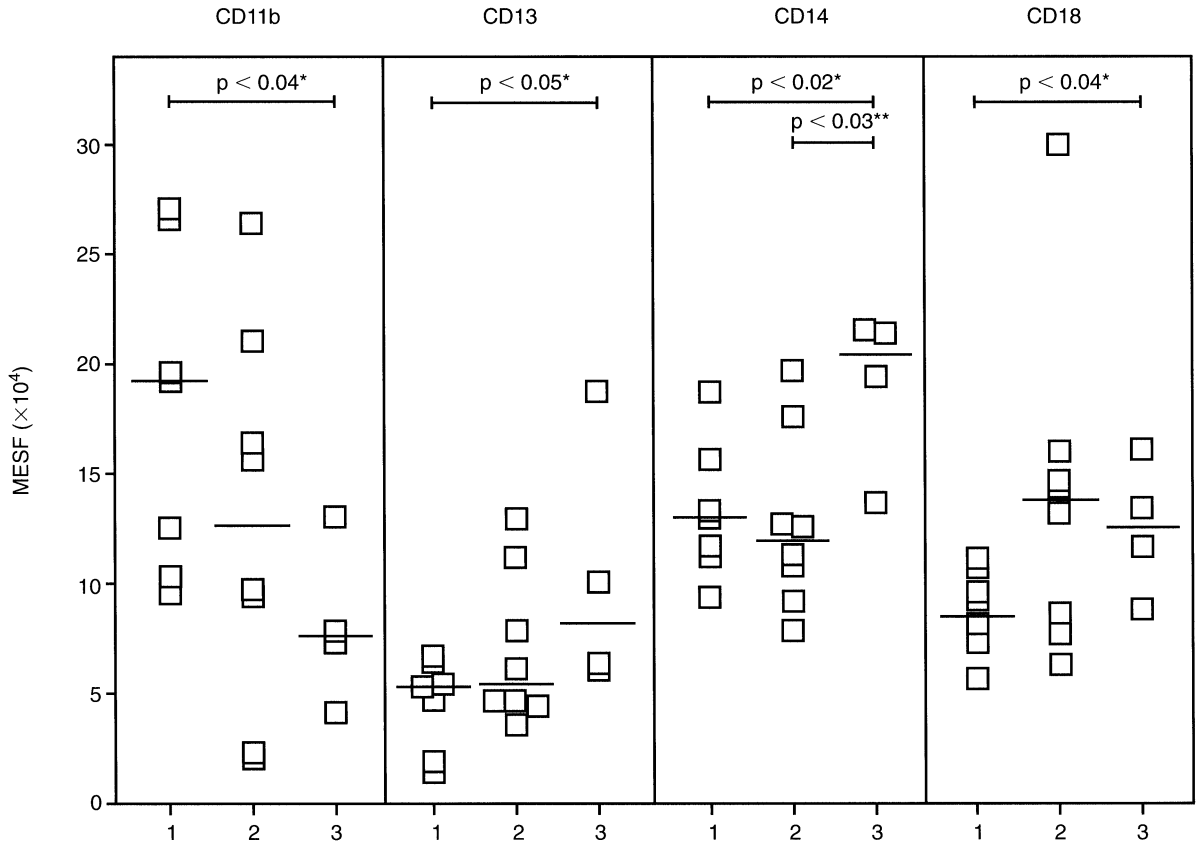

FIG. 1. Expression of CD11b, CD13, CD14 and CD18 cell surface Ag (expressed as MESF $\times 10^4$) by PBM. Median values are represented as a horizontal dash (—). *Value of glucocorticoid group (3) differs significantly from control group (1). **Value of glucocorticoid group (3) differs significantly from anticholinergic/placebo group (2).

CD25 (IL-2R), CD32 (FcγRII) and CD64 (FcγRI) were observed between both groups of patients and healthy controls. The expression of CD16, CD23, and CD25 Ag fell within two-fold the background fluorescence of isotype-matched control moAb, comparable with the expression of RFD9 Ag.

Long-term therapy with inhaled BDP and differences in subsets of peripheral blood lymphocytes

In addition to analysing the cell surface Ag expression on PBM, we investigated whether long-term therapy with inhaled BDP is asso-

ciated with differences in the percentage of T and B lymphocytes in peripheral blood (Table 4). No differences were observed in the percentages of either $CD4^+$ or $CD8^+$ T cells. However, both groups of patients had higher percentages of $HLA\text{-}DR^+$ T cells in their peripheral blood than healthy controls. Treatment with inhaled BDP did not alter the percentage of these activated T cells. Interestingly, patients who received anticholinergic/placebo, but not the patients treated with inhaled BDP, had a significantly higher percentage of B lymphocytes ($CD20^+$ cells) in their peripheral blood compared with healthy controls (Table 4).

Discussion

In this study, we show an almost two-fold higher expression of CD14 Ag on PBM from allergic asthmatics treated for 2.5 years with an inhaled glucocorticoid (BDP, 800 μg daily) compared with patients treated with anticholinergic or placebo. In addition, long-term treatment of asthmatic patients with an inhaled glucocorticoid, but not anticholinergic or placebo, was associated with a significantly reduced expression of CD11b Ag on PBM, and a significantly increased expression of CD13, CD14, and CD18 Ag compared with healthy control subjects.

Glucocorticoids were introduced in 1949 as a new and promising drug in the treatment of inflammatory diseases.[42] Shortly thereafter, local administration of glucocorticoids was commenced to by-pass the unwanted systemic side effects.[43] Nowadays, inhaled glucocorticoids are widely accepted as the treatment of choice in chronic asthma.[28] Several reports showed that inhaled glucocorticoids are safe and almost free of systemic effects. However, high doses of inhaled glucocorticoids may still influence the hypothalamus-pituitary-adrenal axis.[28,44] More evidence for systemic effects of inhaled glucocorticoids comes from studies on circulating eosinophils and T cells. Circulating hypodense eosinophils, which have been shown to exhibit a great inflammatory potential, were reduced in asthmatic subjects after short-term treatment with inhaled glucocorticoids.[45,46] Furthermore, a small but significant reduction in HLA-DR expression by peripheral blood T cells has been reported after 6 weeks of therapy with inhaled glucocorticoids.[27] To our knowledge, modulation of PBM by inhaled glucocorticoids has not been described up till now.

Leukocyte adhesion molecules, among which the CD11/CD18 family of β_2 integrins, play an important role in the recruitment of cells from peripheral blood to the site of inflammation as well as in other immunological and inflammatory processes that require direct cellular interactions.[35,47,48] Both the expression and function of adhesion molecules are augmented in response to inflammatory mediators and cytokines.[49] The reduction in CD11b Ag expression by PBM observed here in asthmatic subjects treated with inhaled glucocorticoid, may have indirectly resulted from the local glucocorticoid-mediated reduction in inflammatory mediators in the lung. Alternatively, systemically absorbed BDP may be directly responsible for the modulation of CD11b Ag expression. In contrast to a reduced expression of CD11b, a slightly but significantly elevated CD18 Ag expression was seen on PBM of asthmatics treated with inhaled glucocorticoid when compared with PBM of healthy control subjects. The CD18 Ag is found on the cell surface in association with one of three different α chains, i.e. the CD11a, CD11b, or CD11c Ag.[47] Since the expression of CD11a and CD11c Ag were not analysed in this study, we do not know whether the observed increase in CD18 Ag expression coincides with an increased expression of CD11a and/or CD11c Ag.

Long-term treatment of asthmatics with inhaled BDP was associated with an increased expression of CD13 Ag by PBM compared with healthy controls. CD13 Ag, a cell membrane-bound aminopeptidase-N, has been shown to play an important role in modulating the activity of regulatory oligopeptides.[36] An in-

Table 4. Lymphocyte subpopulations in peripheral blood

	Control group ($n = 7$)	Anticholinergic/placebo group ($n = 8$)	Glucocorticoid group ($n = 4$)
Lymphocytes			
% $CD20^+$	4.2 (3.1–9.1)[a]	7.2 (3.5–10.1)[b] ($p < 0.04$)	7.6 (3.9–16.4)
% $CD3^+$	67.4 (57.1–82.2)	68.3 (52.6–81.9)	73.5 (53.4–79.0)
% $CD3^+/CD4^+$	34.5 (22.0–44.1)	40.6 (29.1–53.9)	38.0 (32.1–40.9)
% $CD3^+/CD8^+$	23.8 (16.3–30.2)	21.2 (11.3–35.7)	33.0 (13.9–42.7)
% $CD3^+/HLA\text{-}DR^+$	1.0 (0.4–2.1)	2.0 (1.2–9.3)[b] ($p < 0.03$)	5.6 (2.0–8.8)[b] ($p < 0.02$)
Ratio CD4/CD8	1.6 (0.7–2.1)	1.7 (0.8–3.6)	1.2 (0.8–2.6)

[a]Median values (range).
[b]Significantly different from control group.

crease in CD13 Ag expression may therefore enhance the cell's capacity to inactivate harmful inflammatory peptides. In this context, it is noteworthy that CD13 Ag expression by purified PBM increases upon *in vitro* culture in the presence of IL-4, suggesting that this cytokine, like glucocorticoids, has potential anti-inflammatory effects.[37,50] Future studies are needed to determine whether the increased expression of CD13 Ag observed here is accompanied by an increased aminopeptidase-N activity.

The CD14 Ag expression by PBM of patients treated with inhaled glucocorticoid was significantly increased compared with PBM of patients who received anticholinergic/placebo and healthy control subjects. It has been shown that CD14 Ag can function as a receptor for LPS.[38] In addition, a role for CD14 Ag has been implicated in the adhesion of monocytes to cytokine-activated endothelial cells.[51] CD14 Ag expression decreases upon differentiation/maturation of monocytes into alveolar macrophages.[38] This process can be inhibited by glucocorticoids.[41,52] Therefore, it may be that in this study the interference of inhaled BDP with the maturation of PBM manifests itself in the increase of CD14 Ag expression.

In this study, we also show that all asthmatic patients, treated with either inhaled glucocorticoid or anticholinergic/placebo, have significantly increased percentages of HLA-DR$^+$ T cells in their peripheral blood when compared with healthy control subjects. Long-term treatment with inhaled BDP did not reduce the percentage of circulating activated (HLA-DR$^+$) T lymphocytes in both groups of patients. Recently, Wilson *et al.*[27] showed in an uncontrolled study that patients treated for 6 weeks with inhaled BDP showed a reduction in the percentage of activated T cells. Compared with our study, they used a higher dose of BDP and they did not compare their result with patients who did not receive inhaled glucocorticoids. In the present study, we also observed that patients treated with bronchodilator/placebo, but not the patients treated with inhaled BDP, had a higher percentage of B lymphocytes in peripheral blood compared with healthy controls. Therefore, treatment with inhaled glucocorticoid may have influenced the proportion of circulating B lymphocytes in our group of patients.

In summary, our data demonstrate that long-term therapy with an inhaled glucocorticoid coincides with an altered expression of at least one cell surface Ag on PBM of allergic asthmatics. In the treatment of patients clinicians should be aware of these systemic effects of inhaled glucocorticoids. Future studies are needed to determine whether changes in cell surface Ag expression by PBM are part of the anti-inflammatory action of inhaled glucocorticoids, and whether they correlate with the glucocorticoid-induced improvement in FEV_1, PC_{20} and clinical symptoms.

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