

Effects of glucocorticoids on endogenous and transcellular metabolism of eicosanoids in asthma

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Background: Human blood polymorphonuclear cells, which biosynthesize eicosanoids from the 5-lipoxygenase (5-LO) pathway, are likely to be involved in asthma, in which glucocorticoids represent the first line of therapy. Their effects on leukotriene release after a short course of treatment, which have been reported in several studies, are controversial. **Objective:** We sought to investigate whether long-term oral glucocorticoids inhibit lipid mediators from the 5-LO pathway. **Methods:** Twelve normal control subjects, 29 asthmatic subjects, and 50 glucocorticoid-dependent asthmatic subjects were included in the study. Polymorphonuclear cells were studied for endogenous and transcellular metabolism of eicosanoids. **Results:** Total leukotriene B₄ production was significantly lower in cells from glucocorticoid-dependent asthmatic subjects (mean $\pm$ SD, 177 ± 26 ng/10⁷ cells) than in control subjects (406 ± 27), untreated asthmatic subjects (421 ± 34), and asthmatic subjects treated with inhaled glucocorticoids (290 ± 56). When incubated with arachidonic acid, these polymorphonuclear cells released very low amounts of 5(S)- and 12(S)-hydroxy-eicosatetraenoic acid (HETE), whereas endogenous 15(S)-HETE was found in substantial amounts. The transformation of exogenous 15(S)-HETE into 5(S),15(S)-diHETE and lipoxins was significantly more important in untreated asthmatic subjects than in control subjects and glucocorticoid-dependent asthmatic subjects. **Conclusion:** This study showed that long-term oral corticotherapy affects the 5-LO activity and leads to a decrease production of all metabolites in contrast to short-term or inhaled glucocorticoids. This study also questions the site of action of glucocorticoids in regulating the availability of arachidonic acid and potential eicosanoid regulation, as previously held in phospholipase A₂ studies. (*J Allergy Clin Immunol* 2001;107:824-31.)

Key words: Lipoxins, leukotriene B₄, 5-lipoxygenase, glucocorticoids, polymorphonuclear cells, asthma

Bronchial asthma is a disease characterized by reversible airway obstruction, increased airway responsiveness, and airway inflammation. Various cells that migrate from the bloodstream to the bronchial tree are

Abbreviations used

AA: Arachidonic acid
FLAP: 5-Lipoxygenase activating protein
HETE: Hydroxy-eicosatetraenoic acid
LO: Lipoxygenase
LT: Leukotriene
LX: Lipoxin
PLA₂: Phospholipase A₂
PMN: Polymorphonuclear cell
RP-HPLC: Reverse-phase HPLC

sources of local inflammation.¹ The role of eicosanoids derived from the 5-lipoxygenase (5-LO) pathway has been clearly established in asthma.^{2,3}

Blood cells play a major role in inflammatory processes by means of their functions of phagocytosis and secretion and by their sensitivity to chemotactic factors.⁴ Human blood polymorphonuclear cells (PMNs), which are able to biosynthesize eicosanoids from the 5-LO pathway,⁵ are involved in inflammatory disorders.^{4,6} Moreover, neutrophils might be involved in the inflammatory pattern observed in severe asthma.⁷⁻⁹ Previous studies have demonstrated that the 5-LO activity of cells obtained from patients with inflammatory disorders is upregulated compared with the 5-LO activity in the same cell type obtained from control subjects. These cells are able to biosynthesize higher levels of eicosanoids from endogenous arachidonic acid (AA).^{10,11} More recently, we have reported that 5-LO activity was enhanced in alveolar macrophages¹² and PMNs¹³ from untreated asthmatic subjects. In the presence of exogenous 15(S)-hydroxy-eicosatetraenoic acid (HETE), total amounts of lipoxins (LXs) biosynthesized were twice as high as that biosynthesized by the cells from control subjects. We have demonstrated that the activated 5-LO in PMNs from untreated asthmatic subjects allowed them to generate LXs, even in the absence of exogenous precursors.¹⁴ The importance of LX involvement in respiratory diseases has been sustained by previous studies from Serhan,¹⁵ who suggested their chalone-like role in the inflammatory response and their effect in cell regulation.

Glucocorticoids represent the first line of therapy in the management of asthma,¹⁶ and their therapeutic efficiency has been associated with the inhibition of inflammatory processes that involve leukocytes.¹⁷ Although most patients respond to glucocorticoids, there is a subset of severe asthmatic subjects with reduced responsive-

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TABLE I. Clinical characteristics of subjects included in the study

	n	Sex	Age (y)	FEV ₁ (% predicted)	Steroid dose (mg/d)
Control subjects	12	7 F/5 M	37.2 ± 3.4	—	0
Asthmatic subjects					
No treatment	14	4 F/10 M	40.7 ± 11.4	86.1 ± 17.7	0
Short-term treatment	6	3 F/3 M	50 ± 17.8	68.1 ± 10.4*	0*
				90.8 ± 7.8†	80†
Inhaled steroid treatment	9	5 F/4 M	54.0 ± 13.5	76.3 ± 15.6	≤1.0‡
Steroid dependent subjects	50	14 F/36 M	57.1 ± 11.1	68.3 ± 18.8	21.6 ± 13.6

Data are expressed as means ± SD.

*Before treatment with oral steroids.

†After treatment with oral steroids.

‡Inhaled steroids.

ness who are called glucocorticoid-dependent asthmatic subjects because they require long-term systemic glucocorticoids to control their symptoms.¹⁸⁻²⁰

The effects of glucocorticoids on leukotriene (LT) secretions have been reported in several studies performed *in vitro* or *ex vivo* after a short-term exposure.²¹ The level of LTC₄ in plasma was decreased after a treatment with glucocorticoids,²² and oral prednisolone given during acute exacerbations of bronchial asthma decreases plasma LTE₄ levels.²³ The effects on LTB₄ synthesis are more controversial. *In vivo*, 1 week of prednisone therapy reduced LTB₄ released by alveolar macrophages, whereas there was no effect on whole blood leukocytes.²⁴ Glucocorticoids had no effect on the release of LTB₄ by blood PMNs.²⁵ In contrast, PMNs from patients with rheumatoid arthritis released higher amounts of LTB₄ 24 hours after a single dose of methylprednisolone.²⁶ In addition, no inhibition of LTB₄ production was observed in either whole blood or isolated populations of blood cells stimulated with the calcium ionophore A₂₃₁₈₇ after glucocorticoid therapy.²⁷

It may be reasonable to hypothesize that long-term oral therapy does not have the same effect on arachidonate metabolism. Many studies have been performed *in vitro* to investigate the glucocorticoid effect on AA metabolism, but few studies have investigated the *ex vivo* effect of long-term administration. Moreover, no studies reported their effect on LX generation in human PMNs.

The aim of the present study was to investigate endogenous and transcellular metabolism in blood PMNs from glucocorticoid-dependent asthmatic subjects receiving oral glucocorticoids for at least 1 year compared with other groups of subjects: (1) controlled and uncontrolled asthmatic subjects without any treatment or after a short-term treatment with high doses of systemic glucocorticoids; (2) asthmatic subjects with a long-term treatment of average doses of inhaled glucocorticoids; and (3) control subjects. Production was measured in blood PMNs after cell stimulation with A₂₃₁₈₇ alone or incubated with AA or 15(S)-HETE.

METHODS

Patients

Twenty-nine nonsmoking subjects with mild-to-moderate persistent asthma were selected according to the American Thoracic Soci-

ety criteria.²⁸ All had a reversible airway obstruction assessed either by an increase of 12% in FEV₁ after inhalation of 200 µg of salbutamol or a positive carbachol inhalation challenge result. Nine of these patients were controlled and treated with a dose of 1000 µg or less of inhaled beclomethasone dipropionate for at least 6 months, along with 100 µg of salmeterol. Six of these patients had uncontrolled symptoms when entering the study. These patients were treated with a short course of oral methylprednisolone given over 10 days (80 mg/d) and demonstrated at least 15% improvement in their morning prebronchodilator FEV₁, after which their symptoms were controlled. Finally, 14 asthmatic subjects had controlled symptoms without any antiinflammatory treatment.

Control of asthma was defined by the absence of (1) nocturnal symptoms and (2) use of short-acting β₂-agonist during the last 7 days preceding the visit.

Fifty patients with severe glucocorticoid-dependent asthma were included in the study. All these patients had been followed in our outpatient clinic for at least 2 years before the beginning of the present study. They are described as glucocorticoid dependent because we failed to wean them from oral glucocorticoids in the previous 2 years, and they presented recurrent threatening episodes of acute asthma in the past 2 years despite optimal treatment and management, as previously described.^{19,20} They were treated on a regular basis with prednisone (median dose, 21.4 mg) and with 1600 µg or more of inhaled beclomethasone dipropionate, 100 µg of salmeterol, and 10 mg/kg of long-acting theophylline according to the recently published guidelines.

Twelve normal volunteers were enrolled in the study. None of them had any previous history of lung or allergic disease. All of them had normal lung function and negative skin allergy test results. None of the healthy volunteers was using any medication.

The study was performed after informed consent and fulfilled the criteria of the ethics committee of our institution.

PMN preparation

Peripheral blood was recovered by means of venipuncture with heparin (25 U/mL). Cells were isolated by centrifugation of blood samples over isotonic Percoll solutions (Kabi Pharmacia, Uppsala, Sweden),²⁹ with densities of 1.097 g/mL (72%) and 1.086 g/mL (63%), as previously described.¹⁴ Briefly, whole blood was layered over Percoll gradient and centrifuged at 400g for 20 minutes at room temperature. PMNs were recovered in the intermediate layer and were then washed with PBS (Dulbecco pH 7.4). Contaminating erythrocytes were lysed in a buffer solution (NH₄Cl, 130 mmol/L; TRIS, 10 mmol/L; and K₂CO₃, 16 mmol/L). PMNs were then washed, resuspended in PBS with Ca⁺⁺/Mg⁺⁺ (2 and 0.5 mmol/L, respectively), and counted. The purity (95%) was evaluated after cytofluorimetry and May-Grunwald staining. The cell concentration was adjusted to 10⁷ cells/mL. Viability assessed by means of the trypan blue exclusion test was always greater than 95%.

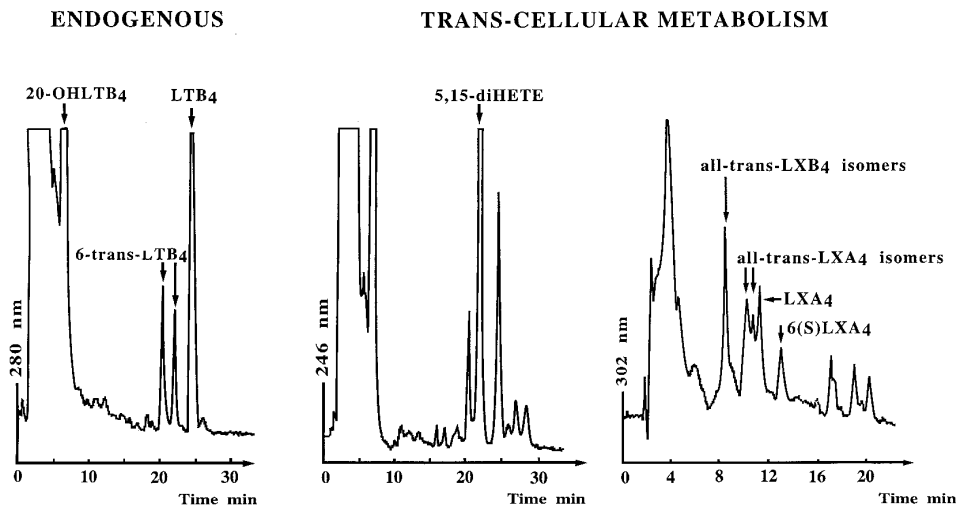


FIG 1. RP-HPLC profile of eicosanoids biosynthesized by PMNs. PMNs (10^7 cells/mL) were stimulated for 5 minutes at 37°C with A_{23187} ($5\text{ }\mu\text{mol/L}$) with and without $15(\text{S})\text{-HETE}$ ($3\text{ }\mu\text{mol/L}$). Peaks were identified by comparison of their retention time with those of standards. **A**, Recording at 280 nm corresponding to the λ_{max} of conjugated trienes. **B**, Recording at 246 nm corresponding to the λ_{max} of $5(\text{S}),15(\text{S})\text{-diHETE}$. **C**, Recording at 302 nm corresponding to the λ_{max} of conjugated tetraenes.

Stimulation procedure

Eicosanoids were generated after cell stimulation with A_{23187} (final concentration, $5 \times 10^{-6}\text{ mol/L}$) for 5 minutes at 37°C , as previously reported.¹¹ At the end of the stimulation, the same volume of cold methanol was added to every sample, which were stored at -20°C until analysis. PMNs from 5 glucocorticoid-dependent asthmatic subjects were incubated with AA (10^{-5} mol/L for 15 minutes). PMNs from 6 control subjects, 14 untreated asthmatic subjects, and 16 glucocorticoid-dependent asthmatic subjects were incubated with $15(\text{S})\text{-HETE}$ and A_{23187} .¹⁴ Simultaneously, the same amounts of AA and $15(\text{S})\text{-HETE}$ were incubated in cell-free medium in the same experimental conditions to insure that eicosanoids did not arise from nonenzymatic lipid peroxidation.

Eicosanoid identification and quantification

Identification and quantification were carried out by using reverse-phase HPLC (RP-HPLC). Before analysis, all the samples were centrifuged to pellet the proteins denatured by methanol addition, and aliquots of the supernatants were directly injected for HPLC analysis. RP-HPLC was carried out by using 2 model 510 pumps (Waters), a U6K injector (Waters), and a programmable multiwavelength detector (model 490, Waters). The data were monitored by using the Millennium 32 chromatography manager (Waters).

DiHETE and triHETE analysis. RP-HPLC was carried out on a Lichrospher 100 RP-18 analytical column ($150\text{ mm} \times 3.9\text{ mm}$, $5\text{-}\mu\text{m}$ particles; Merck). LXs, LTs, and $5(\text{S}),15(\text{S})\text{-diHETE}$ were eluted with methanol/water/acetic acid ($65:35:0.1\text{ [vol/vol/vol]}$), pH 5.7) containing 0.5% EDTA as the mobile phase. They were detected by simultaneously monitoring the wavelengths at 302 , 270 , and 246 nm , corresponding to the λ_{max} of conjugated tetraenes, trienes, and dienes, respectively.

MonoHETE analysis. RP-HPLC was carried out on a Nucleosil C18 column ($250\text{ mm} \times 3.9\text{ mm}$, $5\text{-}\mu\text{m}$ particles) and eluted with methanol/water/acetic acid ($78:22:0.01\text{ [vol/vol/vol]}$) as the mobile phase at a flow rate of 0.85 mL/min with the UV detector set at 237 nm .

Detection and quantification. The amounts of eicosanoids were determined by comparison with standard curves generated by known amounts of authentic standards run in the same conditions in each

HPLC system and are the result of at least 2 separate determinations. The sensitivity threshold was 0.5 to 1 ng at both wavelengths.

Flow cytometry analysis system

The analysis was performed by using the FACScalibur station with Cell Quest data software (Becton Dickinson). 5-LO activating protein (FLAP) and 5-LO polyclonal antibodies from rabbit anti-serum were used as primary antibody, and swine anti-rabbit FITC was used as the secondary antibody. A negative control was performed by using an irrelevant rabbit Ig as the first antibody.

PMNs (10^6) from 6 glucocorticoid-dependent asthmatic subjects were fixed with 4% paraformaldehyde for 20 minutes at room temperature and were permeabilized with saponin buffer (0.5%) to allow the intracellular fixation of the 5-LO or FLAP polyclonal antibodies. After 2 washings, the secondary antibody was incubated in the presence of permeabilizing buffer for 30 minutes at room temperature. Then, after washing, cells were resuspended in $400\text{ }\mu\text{L}$ of PBS and immediately analyzed by using FACScalibur.

Study design

For all patients in each group, a sample cell was analyzed for A_{23187} stimulation. For the AA incubation analysis, the sample cells from 5 of 50 glucocorticoid-dependent asthmatic subjects were studied. For $15(\text{S})\text{-HETE}$ incubation analysis, the sample cells from 6 of 12 control subjects, 14 of 29 untreated asthmatic subjects, and 16 of 50 glucocorticoid-dependent asthmatic subjects were studied. Finally, for 5-LO activity with the flow cytometry analysis system, the sample cells from 6 of 50 glucocorticoid-dependent asthmatic subjects were analyzed.

Statistical analysis

Demographic characteristics are presented as means $\pm$ SD. Results related to eicosanoids are expressed as nanograms per 10^7 PMNs. ANOVA was used for multiple data to compare control subjects ($n = 12$), untreated subjects ($n = 14$), long-term inhaled glucocorticoid-treated subjects ($n = 9$), and glucocorticoid-dependent asthmatic subjects ($n = 50$). Statistical differences were determined by using Kruskal-Wallis and post hoc tests for unpaired data and by using the Wilcoxon W test to compare the 6 asthmatic subjects before and after short-term treatment.

RESULTS

Clinical characterization of patients

The clinical characteristics of subjects are described in Table I. In asthmatic subjects receiving short-term treatment, there was a significant improvement of FEV₁ after treatment. Control volunteers were without any history of allergy or respiratory diseases.

LT biosynthesis

Eicosanoid biosynthesis from endogenous sources. When incubated in vitro with A₂₃₁₈₇, PMNs released 20-OH-LTB₄, LTB₄, and LTB₄ isomers into their culture medium. The elution profile detected at 270 nm showed retention times of 7, 20, 22, and 24 minutes for 20-OH-LTB₄, LTB₄ isomers, and LTB₄ (Fig 1, A).

Eicosanoids produced by the PMNs from glucocorticoid-dependent asthmatic subjects were dramatically decreased, with significant differences between control subjects and asthmatic subjects without any treatment. The amount of 20-OH-LTB₄ was significantly decreased, and amounts of LTB₄ and LTB₄ isomers were often not detectable (Fig 2). 20-OH-LTB₄, LTB₄, and LTB₄ isomers were produced in higher amounts in untreated asthmatic subjects than in control subjects and glucocorticoid-dependent asthmatic subjects. Amounts of eicosanoid released were not different before and after short-term treatment with 80 mg of methylprednisolone (20-OH-LTB₄, 122.00 ± 57.58 vs 140.17 ± 52.84; LTB₄, 75.00 ± 35.98 vs 25.83 ± 10.11; and LTB₄ isomers, 9.83 ± 6.38 vs 34.33 ± 34.33, respectively). The level of LT production was not different compared with patients treated with inhaled glucocorticoids.

HETE biosynthesis from exogenous AA. PMNs from 5 glucocorticoid-dependent asthmatic subjects, which did not generate detectable amounts of 5-HETE after A₂₃₁₈₇ alone, were incubated in the presence of AA (10⁻⁵ mol/L). Very low amounts of 5-HETE or 12-HETE were detected, but in contrast, 15-HETE was biosynthesized in substantial amounts (587.2 ± 99.7 ng/10⁷ PMNs, Fig 3).

Eicosanoid biosynthesis from exogenous 15(S)-HETE. Cells from 6 control subjects, 12 asthmatic subjects, and 12 glucocorticoid-dependent asthmatic subjects were stimulated in the presence of 15(S)-HETE, which was transformed into 5(S),15(S)-diHETE (detected at 246 nm, with a retention time of 22 minutes; Fig 1, B) and trihydroxyderivatives containing conjugated tetraene, all trans-LXB₄ and LXA₄ isomers, LXA₄, and 6S-LXA₄ (detected at 302 nm, with retention times of 9.5, 11.5, 12.5, and 14.5 minutes, respectively; Fig 1, C). The levels of eicosanoids from 15(S)-HETE transformation in glucocorticoid-dependent asthmatic subjects were lower compared with those of untreated asthmatic subjects (LX, 7.20 ± 3.02 vs 54.63 ± 4.73; 5(S),15(S)-diHETE, 37.00 ± 5.18 vs 90.2 ± 4.3, respectively; Fig 4). Moreover, these amounts were lower than in PMNs from control subjects (26.05 ± 1.92 for LX and 60.67 ± 8.09 for 5(S),15(S)-diHETE; Fig 4).

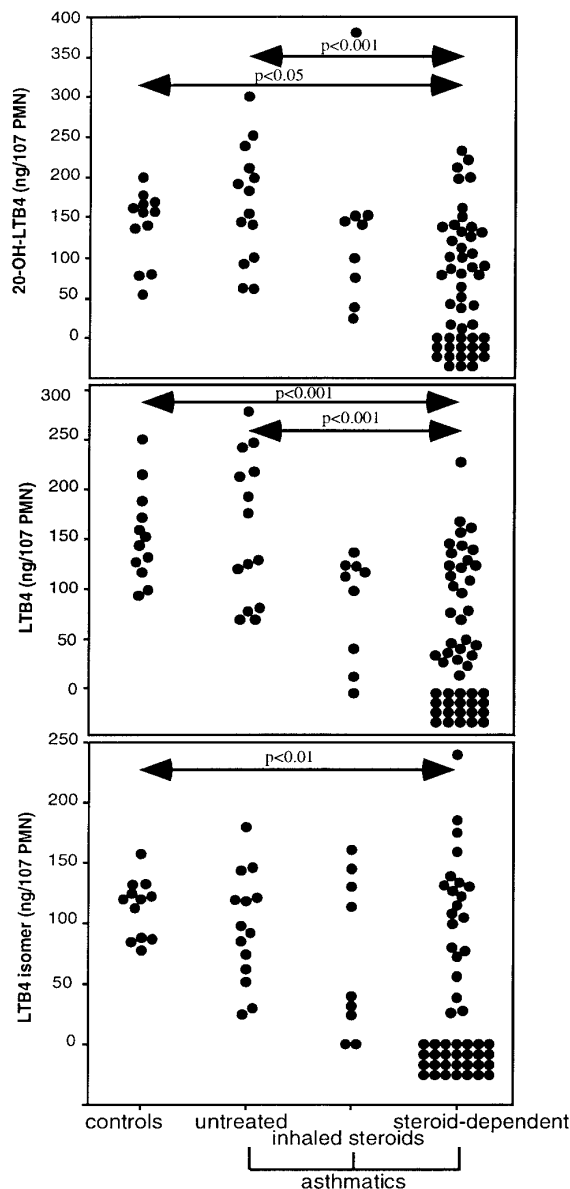


FIG 2. Individual production of 20-OH-LTB₄, LTB₄, and LTB₄ isomers by PMNs. The results are expressed as nanograms of eicosanoids produced for 10⁷ PMNs. The 3 plots report the results related to PMNs from each population of studied patients. Significant differences were observed between glucocorticoid-dependent asthmatic subjects and control subjects or untreated asthmatic subjects for 20-OH-LTB₄ and LTB₄ and between glucocorticoid-dependent asthmatic subjects and control subjects only for LTB₄ isomers (Kruskal-Wallis).

Assessment of the presence of 5-LO and FLAP

Fig 5 shows the representative histogram of one of the 6 experiments performed with the cells obtained from the 6 glucocorticoid-dependent asthmatic subjects. The fluorescence intensity was increased in the presence of antibodies against 5-LO and FLAP compared with the negative control. This result shows that the enzymes were

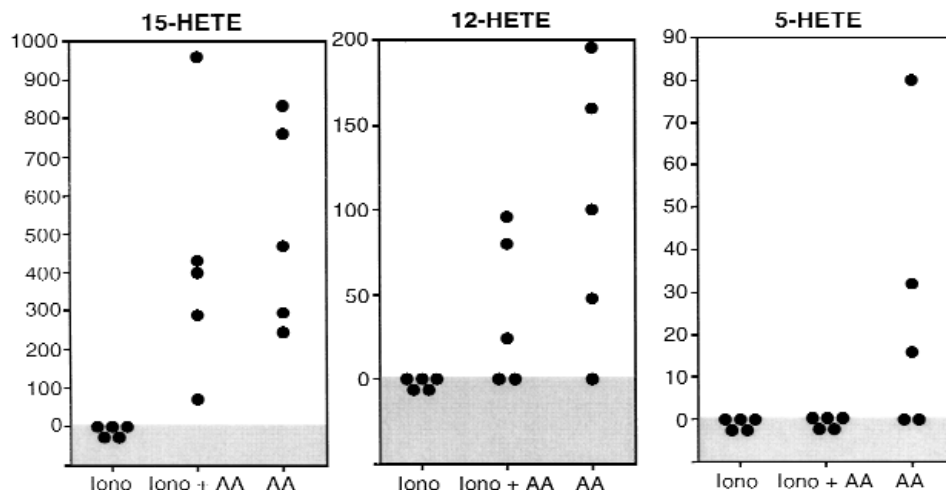


FIG 3. Total HETE metabolites released by PMNs from 5 glucocorticoid-dependent asthmatic subjects. Cells were incubated with exogenous AA for 15 minutes. HETE metabolites were identified by using RP-HPLC analysis on nucleosil C18 column (eluting solvent methanol/water/acetic acid, 78:22:0.01 [vol/vol/vol]) at a flow rate of 1 mL/min. The amount of HETE metabolites was quantified by using an external standard method. Results are presented as nanograms for 10^7 PMNs. The higher levels of HETE metabolites are produced when PMNs were incubated with AA alone, and the main metabolite recovered in PMNs from glucocorticoid-dependent subjects is 15(S)-HETE. *Iono*, A23187.

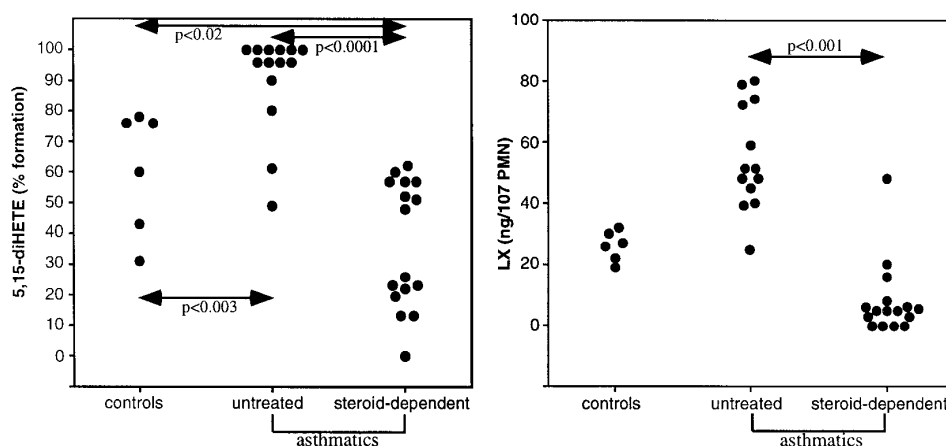


FIG 4. 5(S),15(S)-diHETE and LXs synthesized by blood PMNs from glucocorticoid-dependent asthmatic subjects, untreated asthmatic subjects, and control subjects. PMNs were stimulated with A_{23187} ($5 \mu\text{mol/L}$) for 5 minutes at 37°C with 15(S)-HETE ($3 \mu\text{mol/L}$). The amounts of metabolites were determined by means of RP-HPLC analysis with an external standard method. Results are presented as nanograms per 10^7 cells for LXs and as the percentage of 5(S),15(S)-diHETE formation from the initial added 15(S)-HETE amounts. Significant differences were observed among the 3 groups of patients for 5(S),15(S)-diHETE generation. Significant differences were observed between glucocorticoid-dependent asthmatic subjects and untreated asthmatic subjects for LX generation (Kruskal-Wallis).

both present in the PMNs from glucocorticoid-dependent asthmatic subjects.

DISCUSSION

PMNs from glucocorticoid-dependent asthmatic subjects were investigated for their ability to biosynthesize eicosanoids from endogenous sources or through transcellular metabolism, as compared with PMNs from untreated asthmatic subjects, patients receiving short-

term corticotherapy, and patients receiving long-term inhaled corticotherapy. Sample aliquots were directly injected into the analytical RP-HPLC column without any solid-phase extraction, concentration, or derivatization. The samples only had to be deproteinized and centrifuged, thus minimizing double-bound isomerization, loss, and deterioration of eicosanoids.

Short-term treatment failed to significantly inhibit or decrease LT biosynthesis from endogenous arachidonate in PMNs. No effect was observed for LTB_4 and isomers,

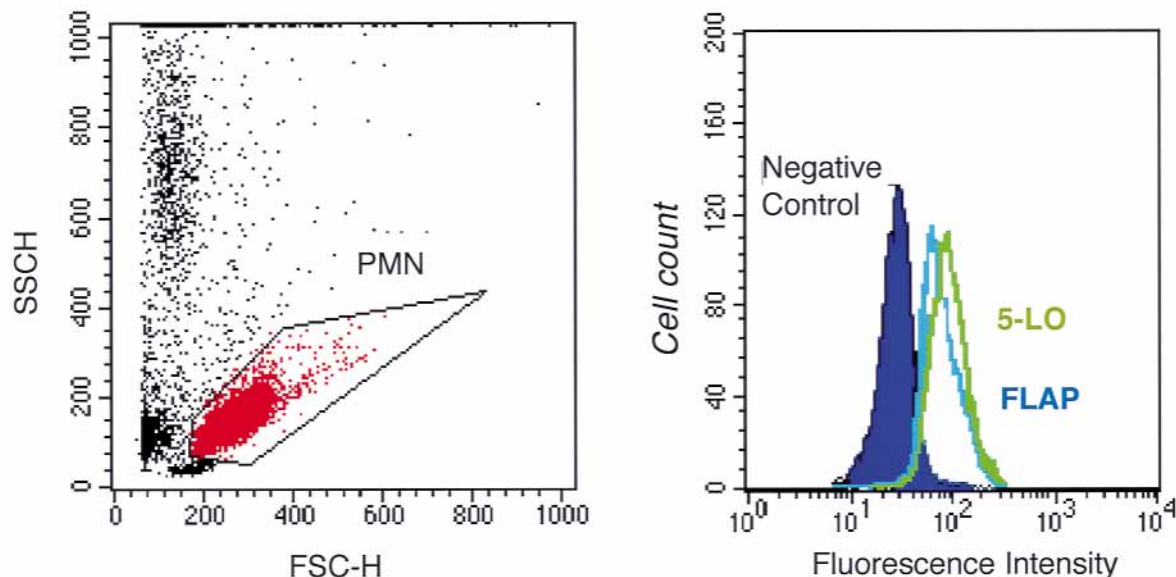


FIG 5. Histogram obtained for the analysis of the presence of 5-LO and FLAP in PMNs from one glucocorticoid-dependent asthmatic subjects. This histogram was representative of the 6 obtained in our study. After fixation and permeabilization, PMNs were incubated with the first specific antibody and then with the second antibody labeled with FITC. The population of PMNs was selected by using a gate for the analysis of the fluorescence. The negative control consists in the replacement of the first antibody with a nonrelevant Ig. The fluorescence was then measured with the 2 antibodies and peaks were compared with the peaks obtained with the negative control. The displacement of the peaks compared with that of the negative control clearly shows the presence of the 2 enzymes. *FSC-H*, ***.

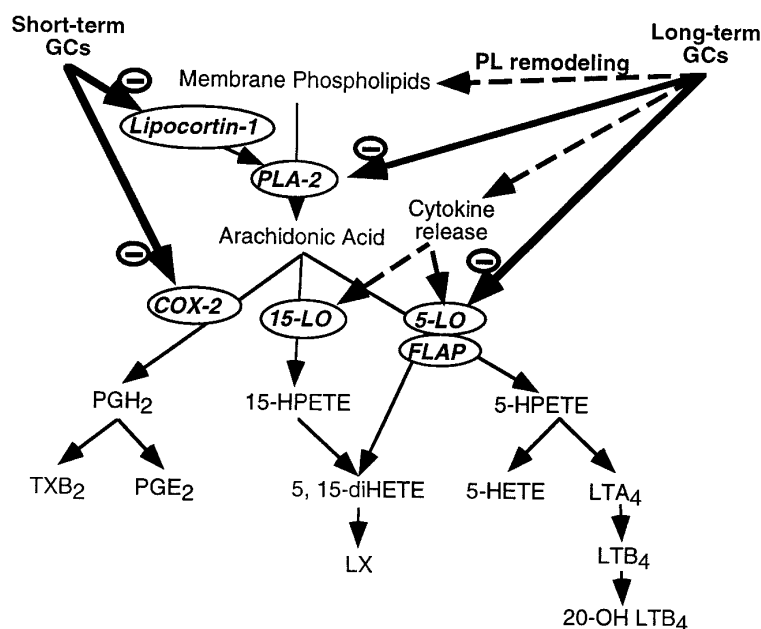


FIG 6. AA metabolism in human PMNs and its regulation by short- and long-term treatment. GC, Glucocorticoid; COX, cyclooxygenase; PG, prostaglandin; TX, thromboxane; HPETE, hydroperoxy-eicosatetraenoic acid.

but in contrast, important variations were observed in 20-OH-LTB₄ levels before and after treatment, showing a tendency toward inflammatory mediator detoxification and normalization toward control levels. These differences were not the consequences of diurnal or weekly

variability because experiments have shown the reproducibility of the results on several analyses of the same subject at intervals of several days or weeks. The lack of inhibition of active LTB₄ production by short-term treatment with prednisone has already been described in puri-

fied leukocytes^{25,26,30} and in whole blood leukocytes.²⁴ Short-term treatment with an oral 80-mg dose of methylprednisolone did not reduce the production of inflammatory mediators by PMNs in the systemic compartment. Concerning long-term inhaled corticotherapy, there are few results in the literature showing its effect on systemic release of mediators. Our results show that long-term inhaled therapy had no effect on LTB₄ metabolites released by blood PMNs. In contrast to short-term and inhaled treatment, long-term oral glucocorticoid therapy has an inhibitory effect in blood PMNs of glucocorticoid-dependent asthmatic subjects. LT production from endogenous arachidonate was inhibited by this long-term treatment. To our knowledge, this is the first time that this aspect of chronic glucocorticoid treatment has been investigated *ex vivo*.

Among inhibitors of eicosanoid biosynthesis, glucocorticoids have been shown to inhibit LT generation by the inhibition of phospholipase A₂ (PLA₂).^{31,32} High amounts of nonpancreatic PLA₂ have been found in serum from patients with inflammatory diseases,³³ suggesting that PLA₂ activity was modulated in blood leukocytes from glucocorticoid-dependent asthmatic subjects. It has been recently observed that *in vitro* treatment of stimulated human eosinophils decrease LTC₄ synthesis. This inhibition might correspond to inhibition of nuclear translocation of cPLA₂.³² Human PMNs have an LTB₄-dependent PLA₂, and the decrease of LTB₄ production could also be explained by eventual PLA₂ inhibition. However, it seems difficult to explain an inhibition of LTs in PMNs from glucocorticoid-dependent asthmatic subjects by PLA₂ inhibition alone because short-term and inhaled corticotherapy are without effect on this inhibition.

All these results showed that glucocorticoid effects could occur early in the AA cascade. Therefore incubations in the presence of AA were performed to evaluate LO activities.^{34,35} In the presence of AA, PMNs from glucocorticoid-dependent asthmatic subjects biosynthesized very low amounts of 5(S)-HETE, whereas they generated high amounts of 15(S)-HETE. There is evidence that free endogenous AA was not available because addition of exogenous substrate triggered other LO activities. LO activity triggered by arachidonate transcellular metabolism was mainly 15-LO rather than 5-LO. PMN 15-LO exists in a cryptic state, and stimuli can convert from an inactive to an active 15-LO, leading to 15-(S)-HETE formation.³⁶ Simultaneously, it has been reported that IL-8 stimulates the formation of 15-HETE by PMNs *in vitro*.³⁷ Blood monocytes of glucocorticoid-dependent asthmatic subjects release very high levels of IL-8.³⁸ These findings could explain why transcellular biosynthesis of AA was primarily used by 15-LO rather than 5-LO in glucocorticoid-dependent PMNs. Our results demonstrate an inactivation of the LT pathway in spite of the presence of 5-LO and FLAP proved with flow cytometric analysis.

PMNs were incubated with 15(S)-HETE to evaluate another means of transcellular metabolism. Use of 15(S)-HETE as an exogenous substrate is interesting to assess

the PMN 5-LO activation independently of endogenous AA availability.¹⁴ PMNs from glucocorticoid-dependent asthmatic subjects were found to metabolize 15(S)-HETE and generate LX and 5(S),15(S)-diHETE but in lesser amounts than in PMNs from control subjects and untreated asthmatic subjects. As reported,³⁹ the decreased capacity to release LX is associated with a clinical phenotype rather than with *in vitro* PMNs stimulation.

Taken together, these results lead to 2 hypotheses concerning enzyme, substrate, or both. Long-term treatment with steroids leads to a lack of activity of 5-LO toward endogenous arachidonate, suggesting that FLAP was activated and that 5-LO translocation may have occurred before *ex vivo* stimulation.⁴⁰ Endogenous arachidonate may be unavailable; this process is suggested by the results of different studies showing that glucocorticoids induced changes in membrane phospholipids.^{41,42} Moreover, it has been reported that glucocorticoids stimulate *in vitro* incorporation of AA into neutral lipids and consequently inhibit its incorporation into phospholipids.⁴³ The localization of endogenous AA storage or the modulation of the deacylation/reacylation pathway in the leukocyte could determine PLA₂ activity. Short-term glucocorticoids may modify the membrane phospholipids, but the pool of remaining AA is sufficient to allow the 5-LO activity. Long-term glucocorticoid use may lead to a complete reorganization of membrane phospholipids, decreasing AA biosynthesis and access by enzymes (Fig 6). This dual effect of short- or long-term systemic glucocorticoids treatment has already been characterized in studies concerning the regulation of the glucocorticoid receptor itself, which is downregulated after a short-term, but not after a long-term, treatment.^{20,38}

This study showed an effect of long-term oral corticotherapy, leading to a decrease in production of AA metabolites from the 5-LO pathway by PMNs from glucocorticoid-dependent asthmatic subjects. Moreover, this study showed, for the first time, that long-term glucocorticoid treatment inhibited the 5-LO activity in PMNs with respect to endogenous arachidonate and to transcellular metabolism, even in the presence of the enzyme. In conclusion, although the role of LTs in the mediation of inflammatory diseases is clearly documented,² the results we have reported contrast with the results obtained with short-term treatment^{24,26,44} and suggest that long-term treatment is acting in part by modifying enzymatic functions of peripheral blood PMNs.

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