A controlled study of 9α,11β-PGF_2 (a prostaglandin D_2 metabolite) in plasma and urine of patients with bronchial asthma and healthy controls after aspirin challenge

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Background: Prostaglandin D_2 (PGD_2) is the predominant cyclooxygenase product of mast cells, the number of which is increased in bronchial asthma. Release of PGD_2 might reflect mast cell activation and disordered function of the asthmatic lung.

Objective: We sought to determine blood and urinary levels of 9α,11β-PGF_2, a major stable PGD_2 metabolite in 2 well-defined phenotypes of asthma, aspirin-induced asthma (AIA) and aspirin-tolerant asthma (ATA), and in healthy control subjects and to study the effects of aspirin on PGD_2 release.

Methods: Using gas chromatography/mass spectrometry, we determined plasma and urinary concentrations of 9α,11β-PGF_2 at baseline in 131 stable asthmatic patients, 65 of whom had AIA and 66 of whom had ATA. Fifty healthy nonatopic subjects served as the control group. The measurements were also performed after an aspirin challenge in 26 of 65 patients with AIA and in 24 of 50 control subjects.

Results: At baseline, patients with AIA had significantly higher plasma levels of 9α,11β-PGF_2 than either patients with ATA or healthy subjects. A similar significant elevation of serum tryptase was observed in patients with AIA compared with patients with ATA and control subjects. Mean urinary 9α,11β-PGF_2 values did not differ among the 3 groups. In patients with AIA, as opposed to healthy subjects, aspirin challenge invariably precipitated a clinical reaction, accompanied in most patients by a further rise in plasma levels of PGD_2 metabolite and tryptase.

Conclusions: In stable AIA, though not in ATA, there is a steady release of PGD_2 into the blood, accompanied by the release of tryptase. Aspirin enhances this reaction in most patients. Release of bronchoconstrictive PGD_2 might contribute to the severe clinical course of AIA. (J Allergy Clin Immunol 2003;111:743-9.)

Key words: *Prostaglandin D_2, 9α,11β-PGF_2, mast cells, aspirin-induced asthma, tryptase, leukotriene E_4*

Abbreviations used

AIA:	Aspirin-induced asthma
ASA:	Aspirin
ATA:	Aspirin-tolerant asthma
COX:	Cyclooxygenase
DP:	Prostaglandin D receptor
GC-NICI-MS:	Gas chromatography–negative-ion chemical ionization–mass spectrometry
GTP:	Guanosine triphosphate
LTE_4:	Leukotriene E_4
NSAID:	Nonsteroidal anti-inflammatory drug

Prostaglandin D_2 (PGD_2) is the predominant cyclooxygenase metabolite of arachidonic acid in mast cells. It is also synthesized by human alveolar macrophages but not by basophils. PGD_2 is released into human airways during acute allergen challenge[1] and causes bronchoconstriction. PGD_2 elicits its biologic effects through interaction with the specific PGD_2 (DP) receptor, a heterotrimeric guanosine triphosphate–binding, protein-coupled, rhodopsin-type receptor.[2] Expression of this receptor in bronchial and alveolar epithelial cells is increased in the asthmatic airway. Mice with a disrupted *DP* gene (DP–/–) do not develop asthmatic attacks in an ovalbumin-induced asthma model.[3] In addition to being produced in the lung, PGD_2 is synthesized in various other tissues in response to allergic stimuli.[4,5] Naturally occurring derivatives of PGD_2 bind to the peroxisome proliferator–activated receptor-γ and stimulate transcription of target genes.[6] Mast cells are often found in close proximity to blood vessels,[7] and these cells accumulate in coronary plaques of infarct-related coronary arteries.[8]

Of the mediators synthesized in mast cells, histamine and tryptase measurements have been introduced into clinical studies. Elevation of their levels in body fluids is considered to reflect mast cell activation.[9,10] As opposed to histamine and tryptase, clinical estimation of PGD_2 has been limited to sporadic measurements, obtained mostly from urine samples.[11-14] In the present study, we focus on blood and urinary levels of the PGD_2 stable metabolite in 2 distinct types of asthma: aspirin-induced asthma (AIA) and aspirin-tolerant asthma (ATA). The kinetics of PGD_2 release in response to aspirin (ASA) challenge in hypersensitive patients has been also addressed.

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TABLE I. Clinical characteristics of the patients

	ATA (n = 66): mean ± SD (range)	AIA (n = 65): mean ± SD (range)	HC (n = 50): mean ± SD (range)
Age (y)	34.6 ± 12.9 (19-70)	41.6 ± 12.4 (17-63)	35.6 ± 9.3 (25-61)
Male/female	27/39	24/41	17/33
Duration of asthma (y)	10.6 ± 9.6 (0.5-47)	9.9 ± 7.1 (1-33)	
FEV_1			
Baseline (mL)	3138 ± 867	2725 ± 783	
Percent predicted	92.5 ± 14.5	84.9 ± 14.3	
Positive skin prick test results (n)	55	29	0
Total IgE, geometric mean ± SD (IU/mL)	129.3 ± 296.8	85.8 ± 297.5	38.8 ± 65.2
Inhaled steroids			
No. of users	45	58	
Dose (μg/d)	1000 (250-2000)	1090 (320-2000)	
Oral steroids			
No. of users	20	25	
Dose (mg/d)	10.6 (2-16)	8.4 (2-20)	

ATA, Aspirin-tolerant asthma; *AIA,* aspirin-induced asthma; *HC,* healthy control subjects.

METHODS

Subjects

Three groups of subjects were studied. The first group consisted of 66 asthmatics who tolerated ASA (ATA). Within the previous 2 years each of them had occasionally used ASA and denied any adverse effects when specifically queried. In 35 of them selected at random, an ASA challenge test was performed; results were negative in all cases. The second group comprised 65 patients with AIA. Their disease developed according to a pattern characterized by a sequence of symptoms.[15] All had had at least 1 episode of asthma attack after ingestion of ASA or other nonsteroidal anti-inflammatory drugs (NSAIDs), and the diagnosis was confirmed by oral ASA provocation tests, performed within 1 year before the study. All patients had stable asthma, and none had experienced an exacerbation thereof or a respiratory tract infection in the 6 weeks preceding the study.

The control group consisted of 50 healthy nonatopic subjects with no history of asthma or allergy. Both skin prick tests to common aeroallergens and a qualitative in vitro assay for determination of specific IgE antibodies were negative in all subjects. They were found to tolerate NSAIDs and had no history of adverse reactions to ASA and other ASA-like drugs. The patients' clinical characteristics are given in Table I.

Oral challenge test with ASA

During the study, an oral challenge test with ASA was performed in 26 of 65 patients with AIA and in 35 of 66 patients with ATA. It was pursued for 2 consecutive days, as previously described,[16] subject to minor modifications. On the first day, placebo was administered, the dose consisting of 2 capsules spaced 2.5 hours apart. FEV_1 was measured every 30 minutes. If variations in FEV_1 of >15% occurred, the patient was excluded from further study because of bronchial instability. On the second day, each patient received increasing doses of ASA (27, 44, 117, and 312 mg) every 2.5 hours until a cumulative dose of 500 mg had been reached. FEV_1 was measured before the first dose and subsequently every 30 minutes. Patients were observed for the following clinical symptoms: bronchospasm, tightness of chest, wheezing, rhinorrhea, nasal congestion, ocular injection, periorbital swelling, and erythema of the skin. The challenge was interrupted if a decrease of ≥20% in FEV_1 occurred, with or without extrabronchial symptoms, or when the maximum cumulative dose of ASA had been reached. The test result was considered positive in the former case and negative in the latter. The cumulative dose causing a 20% fall in FEV_1 was calculated and recorded as PD_{20}. Provocations always started between 8:00 AM and 8:30 AM. Short-acting β_2-agonists were not used 8 hours before the challenge. Long-acting β_2-agonists and theophylline were withdrawn for 24 hours. It was recommended that short-acting antihistamines and cromones not be taken 5 days before the challenge.

In the control group a single dose of 100 mg of ASA, corresponding to the mean PD_{20} for the AIA group, was administered to 10 subjects. Several weeks later the same 10 subjects received a single dose of 600 mg of ASA. The administration of each ASA dose was preceded a day earlier by a single dose of placebo. In another instance, 14 of 50 healthy subjects received 3 doses of ASA within a single day—ie, 100 mg in the morning, 600 mg 5 hours later, and 600 mg after the next 5 hours (total dose of ASA, 1300 mg).

Blood collection

Blood samples were taken at baseline from all subjects. Only from 26 patients with AIA who had undergone the ASA challenge were blood samples obtained before the provocation and at 5, 30, 60, and 120 minutes thereafter, after a fall in FEV_1 of ≥20% was reported. On a placebo day, blood was sampled after administration of the second placebo capsule within the same time intervals as after ASA administration.

From the control subjects, blood samples were taken before and at 15, 30, 60, and 120 minutes after administration of a single dose of ASA or placebo. When 3 doses of ASA or placebo were administered within a single day, the samples were collected before the first dose, 5 hours later (ie, before the second dose of ASA), and subsequently 24 hours after the administration of the first dose.

For 9α,11β-PGF_2 measurement, blood samples were immediately centrifuged at 3500 rpm for 10 minutes. To 1 mL of plasma, 500 pg of deuterium-labeled $PGF_{2\alpha}$ ($[^2H_4]PGF_{2\alpha}$) as an internal standard was added. Samples were immediately processed or stored at –80°C until assayed (not in excess of 2 months).

Urine collection

From all subjects urine samples were collected at baseline after a 2-hour accumulation of urine in the bladder. Only from 26 patients with AIA who were undergoing ASA challenge were urine samples collected at baseline and at 2, 4, and 6 hours after a fall in FEV_1 of ≥20% or after the administration of the second capsule of placebo.

TABLE II. Baseline values of eicosanoids and tryptase in the study groups

				P value		
	AIA	ATA	HC	AIA vs ATA	AIA vs HC	ATA vs HC
Plasma 9α,11β-PGF_2 (pg/mL)	7.39 ± 4.19	5.33 ± 2.97	5.11 ± 1.73	<.01	<.001	>.05
Serum tryptase (μg/L)	7.39 ± 5.86	4.21 ± 1.77	4.25 ± 1.30	<.05	<.05	>.05
Urinary 9α,11β-PGF_2 (ng/mg creatinine)	1.01 ± 0.57	0.91 ± 0.65	1.06 ± 0.51	>.05	>.05	>.05
Urinary LTE_4 (pg/mg creatinine)	1420.9 ± 1185.9	482.8 ± 337.5	336.8 ± 191.1	<.001	<.001	>.05

Values are expressed as means ± SDs.
ATA, Aspirin-tolerant asthma; AIA, aspirin-induced asthma; HC, healthy control subjects.

In 10 healthy control-group subjects, the urine samples were collected in the same manner as for the ASA provocation challenge. The samples were taken before and at 2, 4, and 6 hours after a single dose of ASA or placebo. When 3 doses of ASA or placebo were administered, the urine samples were collected only before the first morning dose and then 24 hours later.

Estimations of 9α,11β-PGF_2, tryptase, and leukotriene E_4

Plasma baseline levels of 9α,11β-PGF_2 were analyzed in all 65 ASA-sensitive asthmatics, in all 50 healthy control subjects, and in 47 of 66 patients with ATA. Serum baseline tryptase levels were measured in 29 of 66 patients with ATA, 29 of 65 patients with AIA, and 24 of 50 healthy control subjects. Postchallenge blood samples for 9α,11β-PGF_2 measurements were obtained from 26 patients with AIA and 24 control subjects, whereas for tryptase measurements, samples were taken from 10 patients with AIA and 24 control subjects. Urinary baseline 9α,11β-PGF_2 and leukotriene E_4 (LTE_4) excretions were estimated in all subjects. Postchallenge samples were collected from 26 patients with AIA and 24 control subjects.

9α,11β-PGF_2 was measured in plasma and urine by gas chromatography–negative-ion chemical ionization–mass spectrometry (GC-NICI-MS) after the extraction stage, through use of a C18 Sep-Pak cartridge, derivatization to pentafluorobenzyl ester, thin-layer chromatography purification, and derivatization to trimethylsilyl ether (Hewlett Packard, Palo Alto, Calif). [$^2H_4$]$PGF_{2\alpha}$ was used as an internal standard in a manner identical to gas chromatography–electron impact mass spectrometry, as reported by Obata et al.[17] Tryptase was measured in serum by a fluoroenzyme-immunoassay method on a UniCAP100 tryptase system (Pharmacia Upjohn Diagnostics, Uppsala, Sweden). LTE_4 excretion was measured in unpurified samples[11] by ELISA (Cayman Chemicals, Ann Arbor, Mich) and expressed in picograms per milligram of creatinine.

Skin tests

Skin prick tests with 14 common aeroallergens (Soluprick, ALK, Hørsholm, Denmark) were performed. The positive control was histamine hydrochloride (1 mg/mL). The diluent for the allergens tested was used as a negative control. A positive skin prick test was defined as a wheal with a mean diameter 3 mm larger than that of the negative control.

Qualitative measurement of specific IgE

A qualitative in vitro assay for the differential determination of specific IgE to allergens was pursued in serum by a fluoroenzyme-immunoassay method through use of the PharmaciaCAP System Phadiatop (Pharmacia Upjohn Diagnostics). The cutoff point for positive and negative results was 0.35 kU/L.

Statistical methods

Significance between 3 groups of subjects under baseline conditions was calculated by 1-way ANOVA for independent variables, followed by the least significant difference test for the paired comparison. The relations between baseline and postchallenge levels were assessed by a repeated-measures ANOVA for dependent variables. Correlation between variables was estimated with the Pearson correlation coefficient. Statistical significance was defined at $P < .05$. Results were expressed as means ± SDs. Statistical evaluation was facilitated by a validated statistical software package, Statistica for Windows (version 5.5; StatSoft, Tulsa, Okla).

The protocol was approved by the local Ethics Committee, and written, informed consent was obtained from each subject.

RESULTS

Clinical observations

In all ASA-sensitive asthmatic individuals undergoing oral ASA challenge, the provocation caused dyspnea and a decrease of FEV_1 of ≥20%. Nasal symptoms in the form of nasal discharge developed in 14 subjects, whereas nasal blockage occurred in 10 of them. Eleven patients experienced itching of the eyes. Facial flush was recorded in 3 patients, headache in 4 patients, and gastrointestinal discomfort in 4 subjects. The symptoms were relieved by short-acting β_2-agonists. Systemic corticosteroids were required in 3 cases only. No severe, life-threatening reactions were observed. Placebo challenge was negative in all patients. In all 35 patients with ATA and in 24 control subjects, the ASA challenge was reported as negative.

Eicosanoids and tryptase baseline values

When mean baseline plasma levels of PGD_2 metabolite were compared, the highest values were recorded in patients with AIA (Table II). The levels measured in patients with ATA and healthy control subjects were similar and significantly lower than those of the AIA group. The increased mean baseline PGD_2 levels were manifested as a constant feature; in 26 patients with AIA who had undergone ASA challenge and had baseline measurements repeated on 2 consecutive days (placebo and ASA days), no significant differences were encountered ($P > .05$). There were no correlations between the dose of systemic corticosteroids and baseline levels of 9α,11β-PGF_2 in patients with either AIA or ATA who were receiving such therapy.

TABLE III. Plasma 9α,11β-PGF_2 and serum tryptase levels at baseline and after ASA challenge

	n	Baseline*	5 min	30 min	60 min	120 min
9α,11β-PGF_2 (pg/mL)						
Placebo	26	6.68 ± 1.97	6.06 ± 2.03	6.19 ± 2.21	6.2 ± 2.09	6.13 ± 1.98
Aspirin	26	6.97 ± 3.27	9.59 ± 6.19†	8.81 ± 5.85	7.7 ± 4.82	6.87 ± 6.87
Tryptase (μg/L)						
Placebo	10	5.82 ± 2.09	5.08 ± 1.97	5.05 ± 1.82	5.14 ± 1.91	5.15 ± 1.67
Aspirin	10	5.85 ± 2.01	7.32 ± 3.47†	8.22 ± 4.81†	8.74 ± 5.31†	10.75 ± 6.61†

Values are expressed as means ± SDs.
*Baseline values calculated for patients in whom postchallenge samples were taken.
†Levels significantly different from baseline ($P < .05$).

TABLE IV. Urinary 9α,11β-PGF_2, and LTE_4 levels at baseline and after ASA challenge

	n	Baseline*	0-2 h	2-4 h	4-6 h
9α,11β-PGF_2 (ng/mg creatinine)					
Placebo	26	1.15 ± 0.37	1.06 ± 0.03	0.99 ± 0.34	0.99 ± 0.38
Aspirin	26	1.10 ± 0.32	1.20 ± 0.65	1.02 ± 0.37	1.15 ± 0.36
LTE_4 (pg/mg creatinine)					
Placebo	26	1416.0 ± 1221.4	1409.1 ± 1060.6	1388.7 ± 1040.1	1332.2 ± 1041.1
Aspirin	26	1495.8 ± 1181.6	2447.6 ± 2597.4†	5473.3 ± 6035.7†	7743.3 ± 9922.7†

Values are expressed as means ± SDs.
*Baseline values calculated for patients from whom postchallenge samples were taken.
†Levels significantly different from baseline ($P < .05$).

Patients with AIA showed the highest baseline tryptase values in comparison with the other 2 groups (Table II). There were no significant differences between the levels measured in the ATA group and the levels measured in the control subjects with respect to this metabolite.

There were no differences in baseline urinary excretion of 9α,11β-PGF_2 in any of the 3 groups (Table II). In patients with ATA the mean baseline level of urinary LTE_4 excretion was not significantly different from that in healthy control subjects (Table II). Patients with AIA had the highest levels of urinary LTE_4, significantly different from the values recorded in both the ATA group and the healthy control group (Table II).

Plasma 9α,11β-PGF_2 after ASA challenge

In ASA-sensitive asthmatic patients, ASA caused a significant, early mean 1.4-fold rise in 9α,11β-PGF_2 level in blood in comparison with the prechallenge value ($P < .05$; Table III). At the later time points (30 and 60 minutes) the levels showed a tendency to higher readings in comparison with baseline values, though the differences failed to reach statistical significance ($P > .05$). In individual patients the response was not homogeneous. The rise (1.4- to 7-fold) was observed in 15 patients, there was no change in 7, and a decrease (by 1.4- to 2.5-fold) was seen in 4 subjects. After placebo administration, no significant differences were found between the mean baseline value and the values measured at all sampling time points (Table III). ASA administered in different doses to healthy volunteers did not affect the post-ASA blood levels of 9α,11β-PGF_2.

Serum tryptase after ASA challenge

ASA provocation was followed by a gradual significant rise in serum tryptase levels, which began 5 minutes after the challenge ($P < .05$) and reached the peak 120 minutes after the provocation ($P < .01$; Table III). Such positive response occurred in 8 of 10 patients from whom postchallenge samples for tryptase measurements were collected. Placebo had no effect (Table III).

Urinary 9α,11β-PGF_2 after ASA challenge

After the ASA challenge, at no time did the mean values show statistically significant differences compared with baseline (Table IV). Measurements in individual patients revealed a moderate rise (1.3- to 2.6-fold) in 11, no change in 7, and a fall (by 1.3 to 1.7) in 8. Likewise, placebo administration did not cause any significant differences in urinary levels of this metabolite (Table IV). Ingestion of different doses of ASA did not affect the urinary 9α,11β-PGF_2 excretion in healthy control subjects.

Urinary LTE_4 after ASA challenge

All patients with AIA who were subjected to the ASA provocation test responded to the challenge with a rise in urinary LTE_4 excretion. The peak values increased by 1.4 to 23 times over the baseline values. The mean LTE_4 value was found to have risen by 1.7-fold over baseline in the samples collected between 0 and 2 hours ($P < .05$; Table IV). It continued to rise and reached its peak in the last sample collected (ie, 4 to 6 hours after the ASA challenge); it was 5-fold higher in comparison with baseline

($P < .01$; Table IV). The levels measured before and after placebo administration were relatively stable and did not differ statistically (Table IV).

In the group of healthy control subjects, LTE_4 urinary excretions showed no statistical differences at any of the sampling times compared with baseline values, irrespective of the ASA dose actually administered.

Analysis of correlation between the variables studied

No correlation was found between the following variables: (1) plasma and urinary levels of 9α,11β-PGF_2; (2) plasma 9α,11β-PGF_2 and serum tryptase levels; and (3) urinary LTE_4 and urinary 9α,11β-PGF_2 levels, before and after ASA challenge.

DISCUSSION

Because of rapid metabolism and artifactual generation of prostaglandins during sampling and isolation of plasma, it is widely recognized that measuring metabolites rather than the parent compound is by far the most efficacious method of assessing the endogenous production of prostaglandins.[18,19] The predominant pathway of PGD_2 metabolism has been shown to involve transformation of PGD_2 by the 11-keto-reductase enzyme to 9α,11β-PGF_2. This metabolite does in fact boast biologic activity. It is equipotent with PGD_2 in causing constriction of human airways in vitro and in vivo,[20] it contracts isolated human coronary arteries,[21] and it inhibits platelet aggregation.[22] The mass spectrometry measurement of PGD_2 metabolites in blood is highly accurate and sensitive, though not very efficient, and therefore requires expensive instrumentation. Very limited data are available on 9α,11β-PGF_2 levels in human blood. In 3 asthmatic children,[17] plasma levels of 9α,11β-PGF_2 were lower at the time of discharge from hospital in comparison with those on admission, though they all remained within the range for healthy subjects. In the case of acute urticaria and bronchospasm of unknown origin,[19] the level of 9α,11β-PGF_2 was 5 pg/mL—ie, in the upper range for normal individuals studied by the authors (average, 3 pg/mL). A very high level (143 pg/mL) was recorded in single cases of mastocytosis.[19,23]

In a group of patients with AIA assessed in the present study, the mean baseline 9α,11β-PGF_2 plasma levels were significantly higher than in both the patients with ATA and the healthy control subjects. The elevation of this biomarker appeared to be a constant feature, because it was recorded in the blood sampled on 2 consecutive days in the same group of subjects with AIA. The most likely cellular sources of PGD_2 were mast cells, which infiltrate airway smooth muscle in asthma.[24] It was then interesting to note that the mean baseline levels of serum tryptase, another marker of mast cell activation, also showed the highest values in the AIA group in comparison with the other 2 groups. A rise in PGD_2 and tryptase levels at baseline suggests an ongoing process of mast cell and/or macrophage activation in stable AIA. It also indicates that in AIA, a second arachidonate metabolite, apart from cysteinyl leukotrienes, is overproduced. Exacerbation of asthma can lead to a rise in plasma PGD_2. Hence, in a group of asthmatic patients, including those with AIA, 9α,11β-PGF_2 plasma levels were significantly higher at exacerbation than at recovery.[25] A striking increase of eosinophils in AIA airways[26] might therefore account for the ongoing mast cell activation. Eosinophils have the capability to generate and excrete stem cell factor. This is the only factor essential for human mast cell development and is a powerful direct activator of the mediator release by mature mast cells.[27]

ASA challenge performed in patients with AIA caused an early 1.4-fold increase in plasma 9α,11β-PGF_2, which was short-lasting, albeit statistically significant. The response was not homogeneous. Only approximately 65% of the subjects responded with an evident increase in PGD_2 metabolite. Such a nonhomogeneous response was observed previously after an ASA challenge, when PGD_2 was measured in bronchoalveolar lavage fluid[28] or in urine.[29] Two isoforms of cyclooxygenase (COX) coexpress in the same cell types, but their proportions can vary.[30] This different proportion might affect the extent of COX inhibition by ASA, a nonselective inhibitor. Specific COX2 inhibitors do not trigger cysteinyl leukotrienes and PGD_2 release in the patients with AIA.[31] The concomitant increase in serum tryptase levels after ASA challenge, as observed in patients with AIA assessed in the present study, provides additional support for the assumption that in this type of asthma, mast cells are activated by ASA, as already suggested by other authors.[32] This is also consistent with the results of our previous study[33] of a group of 10 patients with AIA, in whom the mean serum tryptase level was found to increase significantly within 4 hours of ASA ingestion. Results obtained by others[34] were far from equivocal; marked elevations of tryptase levels in post-ASA serum samples were observed in only 3 of 17 subjects studied.

In urine, as opposed to plasma, baseline 9α,11β-PGF_2 levels were similar in the 3 groups studied, as earlier reported.[12] In the AIA group investigated in the present study, ASA provocation did not cause evident changes in postchallenge values of this metabolite. This result is in opposition to the observations of O'Sullivan et al[12] and Mita et al,[13] who reported an increase in urinary 9α,11β-PGF_2 after ASA challenges in patients with AIA. Different routes of administration (inhaled[12] or intravenous[13]) and different assay methods might well account for these discrepancies. The authors of both of the aforementioned studies used the same enzyme-immunoassay method, whereas the GC-NICI-MS method was applied in the present study. The former measures the sum of 9α,11β-PGF_2 and 2 isomers of its dinor metabolite, whereas the latter is restricted to the detection of 9α,11β-PGF_2.[35] Hence, when a major PGD_2 urinary metabolite,[36] PGD-M (9α,11β dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20 dioic acid), was determined by GC-NICI-MS in patients with AIA after the ASA challenge, the mean levels of this metabolite remained unaltered, although in 6

of 9 patients studied, a mild rise occurred.[29] Apart from 9α,11β-PGF_2 and PGD-M, other PGD_2 metabolites are excreted in urine.[37] Various activities of the enzymes that metabolize PGD_2 in blood and tissues might therefore account for the lack of correlation between plasma and urinary levels of the PGD_2 metabolite investigated in the present study.

PGD_2 released in patients with asthma might affect the clinical presentation of asthma through its bronchoconstrictive action and potent chemoattractive properties for T_H2 lymphocytes, basophils, and eosinophils.[38] The role of PGD_2 degradation products via nonenzymatic chemical modification (eg, 15-deoxy PGJ_2) and their interaction with proliferator-activated receptors remains a matter of controversy.[39] Half of patients with AIA require long-term oral corticotherapy to control their asthma.[40] A steady mast cell activation with PGD_2 release might be one of the key reasons for the severe course of this particular type of asthma.

Different mechanisms might operate in other phenotypes of asthma. Pretreatment with indomethacin prevented an increase in a stable PGD_2 urinary metabolite but did not affect the airway response to the inhaled allergen in atopic asthmatic patients.[41] COX inhibition by indomethacin, however, might also prevent synthesis of "beneficial" prostaglandins—ie, PGE_2[42]—or cause an increase in the amount of constrictor 5-lipooxygenase products formed by activated cells.[43]

No correlation was found in the present study between urinary LTE_4 and plasma or urinary PGD_2 metabolite at baseline or after the ASA challenge. These complex issues await further studies.

It was surprising to find that in healthy subjects, even the high doses of ASA failed to diminish blood and urinary levels of the stable PGD_2 metabolite. This is in contrast with the inhibitory effects of ASA on in vivo biosynthesis of other prostanoids, such as prostacyclin and thromboxane A_2,[44] and might point to an unknown regulatory mechanism in COX-mediated production of PGD_2.

As evidenced by the present study, in stable AIA as opposed to stable ATA, there is a steady release of PGD_2 into blood. This implicates a continuous activation of mast cells and/or macrophages, which is further corroborated by the concomitant elevation in baseline levels of tryptase. A specific trigger, such as ASA, enhances the ongoing release of both of these substances in the majority of, though not in all, patients with AIA. Enhanced PGD_2 release might be related to a severe clinical course of AIA. The clinical value of plasma 9α,11β-PGF_2 measurements warrants further studies.

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