

Original article

Possible involvement of mast-cell activation in aspirin provocation of aspirin-induced asthma

Background: Although there is increasing evidence of the importance of cysteinyl leukotrienes (LT) as mediators of aspirin-induced bronchoconstriction in aspirin-sensitive asthma, the cellular origin of the LT is not yet clear.

Methods: Urinary concentrations of leukotriene E₄ (LTE₄), 11-dehydrothromboxane B₂, 9 α ,11 β -prostaglandin F₂, and N⁷-methylhistamine were measured during the 24 h following cumulative intravenous administration of increasing doses of lysine aspirin to asthmatic patients. In addition, the urinary concentrations of these metabolites were measured on 5 consecutive days in a patient who suffered an asthma attack after percutaneous administration of nonsteroidal anti-inflammatory drugs.

Results: In aspirin-induced asthma patients (AIA, *n* = 10), the basal concentration of urinary LTE₄, but not the other metabolites, was significantly higher than that in aspirin-tolerant asthma patients (ATA, *n* = 10). After intravenous aspirin provocation, the AIA group showed a 13.1-fold (geometric mean) increase in excretion of LTE₄ during the first 3 h, and 9 α ,11 β -prostaglandin F₂ also increased in the AIA group during the first 0–3 h and the 3–6 h collection period. N⁷-methylhistamine excretion was also increased, but to a lesser degree. Administration of aspirin caused significant suppression of 11-dehydrothromboxane B₂ excretion in both the AIA and ATA groups. When the percentage of maximum increase of each metabolite from the baseline concentrations was compared between the AIA group and the ATA group, a significantly higher increase in excretion of LTE₄, 9 α ,11 β -prostaglandin F₂, and N⁷-methylhistamine was observed in the AIA group than the ATA group. An increased excretion of LTE₄ and 9 α ,11 β -prostaglandin F₂ has been detected in a patient who suffered an asthma attack after percutaneous administration of nonsteroidal anti-inflammatory drugs.

Conclusions: Considering that human lung mast cells are capable of producing LTC₄, prostaglandin D₂, and histamine, our present results support the concept that mast cells, at least, may participate in the development of aspirin-induced asthma.

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Key words: aspirin-induced asthma; leukotriene E₄; 11-dehydrothromboxane B₂; mast cells; 9 α ,11 β -prostaglandin F₂.

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Ingestion of aspirin or other drugs that inhibit the cyclooxygenase of arachidonic acid elicits respiratory, gastrointestinal, and dermatologic symptoms in a subset of adult asthmatic patients (1, 2). The prevalence of aspirin sensitivity in asthma is estimated to be 10–20%. The mechanism of aspirin-sensitive asthma remains unresolved. However, the data accumulated so far suggest the involvement of peptidyl-leukotriene (LT)s as mediators of aspirin-induced bronchoconstrictor responses. We may summarize the evidence as follows:

1) Patients exhibiting aspirin sensitivity (AIA) have significantly higher basal levels of production of urinary leukotriene E₄ (LTE₄) than non-aspirin-sensitive asthmatics (ATA) (3–6).

- 2) Administration of aspirin resulted in an increase in urinary LTE₄ concentration in the AIA group, although the mechanism of the augmented production of LTC₄ is not known (7, 8).
- 3) Several studies have demonstrated that LT antagonists or 5-lipoxygenase inhibitors attenuate aspirin-induced responses (9–11).
- 4) Immunohistochemical studies of bronchial biopsies indicate that expression of LTC₄ synthase is increased in the AIA group (12), and the existence of a polymorphism at the promoter region in LTC₄ synthase has been proposed as the probable mechanism of the higher expression of LTC₄ synthase (13, 14).

Therefore, it appears that peptidyl-LTs are important and perhaps could even be the predominant mediators of aspirin-induced airway obstruction in the AIA group. However, the cellular source of peptidyl-LT is not known, and it has been demonstrated that eosinophils and mast cells may contribute to aspirin-induced reactions (15, 16). If mast cells are involved in the development of aspirin-induced bronchoconstriction, it may be possible to detect increased levels of the urinary metabolites of mast cell-associated mediators.

Several biochemical markers have been used to implicate the activation of mast cells in disease states and in the biochemical diagnosis of disease associated with the activation of mast cells. Histamine is released after activation of mast cells and therefore measurement of histamine in plasma and the metabolite, N^{ϵ} -methylhistamine (methylhistamine), or N^{ϵ} -imidazoleacetic acid, in urine has been used to assess endogenous mast-cell activation (17–20); however, it is possible that elevated levels of histamine can also result from the activation of basophils. In addition, food intake and ingestion of histamine itself can be another source of increased levels of histamine in plasma and methylhistamine in urine (21). Prostaglandin D_2 (PGD_2) has been identified as a major metabolite resulting from the cyclooxygenase of arachidonic acid in mast cells (22, 23). After generation in the body, PGD_2 is degraded by PGD_2 11-ketoreductase to $9\alpha,11\beta$ -prostaglandin F_2 ($9\alpha,11\beta$ - PGF_2), which is subsequently excreted in urine (24–27). LTC_4 is also generated by activation of human mast cells, although there is a heterogeneity with respect to LTC_4 generation in a subset of human mast cells. Unlike human mast cells present in the skin, lung mast cells generate significant quantities of LTC_4 (22). Once secreted, LTC_4 is metabolized sequentially to LTD_4 and LTE_4 . The urinary excretion of LTE_4 is believed to provide a reasonable reflection of whole-body LTC_4 production in man (28, 29). Clinical evaluation of these events, which suggests mast-cell activation when only a single biochemical marker is used, may provide misleading results, whereas results obtained from the use of multiple biochemical markers may be interpreted with greater confidence. In this study, we measured the concentrations of LTE_4 , $9\alpha,11\beta$ - PGF_2 , and methylhistamine to evaluate mast-cell activation in an AIA group after provocation with aspirin, and the results have been compared with those observed in an ATA group. We also measured levels of 11-dehydrothromboxane B_2 ($DTxB_2$), which is a urinary metabolite of platelet-derived TxA_2 (30–32), the values of which provide objective evidence of cyclooxygenase inhibition.

Material and methods

Chemicals

Reagents were purchased as follows: 4-hydroxy-TEMPO (Sigma, USA); peptidyl-LT enzyme immunoassay kit (Amersham, UK);

$DTxB_2$ radioimmunoassay kit (New England Nuclear, USA); $9\alpha,11\beta$ - PGF_2 enzyme immunoassay kit (Cayman, USA); and methylhistamine radioimmunoassay kit (Pharmacia, Sweden).

Patients and study design

Aspirin provocation test. After informed consent was obtained, urine specimens were collected when asthmatic patients underwent the provocation test to check sensitivity to aspirin. All patients were in a stable condition at the time of the provocation test, and their forced expiratory volume in 1 s values ($FEV_{1.0}$) were higher than 70% of the predicted value in all patients except for one patient in the ATA group (no. 6). All medications were stopped for at least 12 h prior to the provocation test. The clinical characteristics of these patients are shown in Table 1. Statistical evaluation revealed no difference between the groups in age, duration of asthma, and baseline spirometry.

At the beginning of the study, urine was collected and pulmonary function tests were performed at about 9 AM. After intravenous administration of 1 ml of saline, if $FEV_{1.0}$ did not change by more than 10% from the prechallenge baseline, doubling doses (25, 50, 100, and 200 mg equivalent to aspirin) of lysine aspirin were intravenously administered. $FEV_{1.0}$ was recorded every 10 min after the administration, and the interval between doses was 30 min. Provocation was stopped at the appearance of a positive reaction, or when the highest dose of lysine aspirin had been administered. A positive reaction in the airways was defined as when the $FEV_{1.0}$ fell 20% or more from the baseline.

After provocation, the urine excreted during the following periods of time was collected for measurement of eicosanoids: the hour immediately prior to and following the provocation test and the periods 0–3, 3–6, 6–9, and 9–24 h. Samples were collected in polypropylene bottles containing 4-hydroxy-TEMPO, and the aliquots were stored at $-35^{\circ}C$ until analysis.

Patient who developed asthmatic symptoms after percutaneous administration of nonsteroidal anti-inflammatory drugs. A 36-year-old female asthmatic patient, who was already aware of her sensitivity to nonsteroidal anti-inflammatory drugs, accidentally used adhesive plasters containing flurbiprofen and about 1–2 g of an ointment containing 1% of indomethacin for self-treatment of muscle pain. Within 6 h, asthmatic symptoms developed, and she was taken to the emergency room by an ambulance. By 6 h after d.i.v. administration of 10 mg of dexamethasone and 750 mg of theophylline, she had recovered. Urine collection began immediately after signs of improvement and was continued over the next 4 consecutive days. The urine was stored as described above.

Quantitation of urinary metabolites

LTE_4 was quantitated by enzyme immunoassay after purification with high-performance liquid chromatography, as reported previously (33). $DTxB_2$ was measured by radioimmunoassay after purification with reverse phase column by the method of Riutta et al. (34). Methylhistamine was quantitated by radioimmunoassay according to the manufacturer's instructions. $9\alpha,11\beta$ - PGF_2 was quantitated by enzyme immunoassay after extraction with an Empore C18 cartridge, by the procedure reported by O'Sullivan et al. (35). Recovery of $9\alpha,11\beta$ - PGF_2 after purification was approximately 60–70%, when 40–200 pg of authentic standard was added to 0.5 ml of urine. The concentrations were expressed as pg per mg of creatinine.

Statistical analysis

All data were expressed as mean $\pm$ SD, and statistical analysis was performed with the Mann-Whitney U-test. A P value of <0.02 was considered to be statistically significant.

Table 1. Characteristics of patients

Patient no.	Sex	Age (years)	Atopy	Onset of asthma (age in years)	Aspirin sensitivity	Cumulative dose of lysine aspirin (mg)	FVC (l)	FEV _{1.0} (l)	%FVC (l)	PEFR (l/s)
1	F	52	Yes	39	No	375	2.63	2.02	102.3	3.32
2	M	57	No	41	No	375	3.24	1.9	93.1	3.8
3	F	33	Yes	31	No	375	2.54	2.32	87.2	5.76
4	F	21	Yes	4	No	375	3.23	2.7	108.3	6.91
5	F	53	Yes	48	No	375	2.47	2.07	100	6.06
6	F	50	Yes	32	No	375	1.89	1.3	72.9	4.42
7	F	68	No	66	No	375	2.23	1.75	95.2	5.31
8	F	70	No	44	No	375	1.98	1.4	97	5.14
9	M	42	No	?	No	375	4.07	3.75	101.7	11.62
10	F	22	No	?	No	375	2.87	2.87	89.1	6.67
11	F	50	No	42	Yes	25	2.88	1.5	110.7	1.54
12	F	37	No	35	Yes	25/75	3.12/3.19	2.75/2.82	108.3/110.7	7.36/7/32
13	F	79	No	76	Yes	75	1.55	1.32	72.0	4.61
14	M	58	Yes	43	Yes	75	3.34	2.40	86	6.79
15	F	51	Yes	43	Yes	75	3.04	2.4	125.1	6.01
16	M	32	Yes	22	Yes	75	4.01	2.90	98.8	5.93
17	M	26	Yes	21	Yes	75	4.73	3.85	108.7	7.20
18	F	71	Yes	40	Yes	75	2.66	1.96	123.7	4.51
19	F	49	No	37	Yes	75	3.56	2.66	140.2	5.43
20	F	50	Yes	30	yes	75	2.03	1.61	72.5	4.42

Results

The baseline characteristics of the two groups of subjects are presented in Table 1. There was no significant difference between the AIA and ATA groups in age, duration of asthma, and baseline spirometry. The AIA group developed a greater than 20% decrease in FEV_{1.0} after administration of either 25 or 50 mg of aspirin, which corresponds to a cumulative dose of 75 mg, while the ATA group cumulatively received 325 mg of aspirin without significant change in FEV_{1.0}. Thus, the dosage of aspirin was significantly different between the two groups.

Intravenous administration of lysine aspirin was used as an alternative to oral aspirin challenge or the bronchoprovocation test for the screening of aspirin sensitivity. The AIA group exhibited bronchoconstriction with a decrease in FEV_{1.0} of 25–30% within 20 min. Symptoms were restricted to the bronchopulmonary tract, and systemic reaction was not observed in the AIA and ATA groups.

The AIA group had significantly higher basal urinary LTE₄ concentrations than those of the ATA group (67.9 ± 53.0 vs 298.5 ± 388.7 pg/mg creatinine; $P = 0.0056$), although there was a substantial overlap of LTE₄ concentrations between the AIA and ATA groups. In contrast, no significant difference was observed in the concentrations of 9 α ,11 β -PGF₂ (181.2 ± 197.0 vs 168.6 ± 232.5 pg/mg creatinine), DTxB₂ (1340.0 ± 987.6 vs 1013 ± 748 pg/mg creatinine), and methylhistamine (214.3 ± 119.8 vs 136.6 ± 42.7 ng/mg creatinine) between the two groups.

After aspirin provocation in the AIA group, urinary LTE₄ concentrations increased by 13.1-fold (geometric mean; range, 4.7- to 115.4-fold) from the baseline

concentration. The maximum increase occurred in the first 3 h and during the collection interval of 3–6 h after provocation. After aspirin administration to the ATA group, the concentration of LTE₄ excreted in urine was variable and not significantly different from that observed at the baseline time period. When provocation was repeated in one AIA subject (no. 12), after an interval of about 2 months, a very similar pattern of urinary LTE₄ increase was observed in which the maximum increases above the baseline concentration were 24-fold and 19-fold in the subsequent provocation test. Administration of aspirin resulted in a similar, but slightly lower increase in 9 α ,11 β -PGF₂ excretion at the 0–3- and 3–6-h periods (Table 2). When percent change exceeding the mean plus 2 SD for ATA subjects was regarded as a cutoff level, the increase of 9 α ,11 β -PGF₂ was found for 8/10 AIA subjects at 0–3 h; i.e., the concentration of 9 α ,11 β -PGF₂ increased more than 186.9% from the baseline in eight patients. A small increase of methylhistamine also occurred after administration of aspirin in the AIA group. A significant increase of urinary concentration of methylhistamine was observed in the ATA group at 6–9 h after provocation, a finding which may be due to physiologic fluctuation.

TxA₂ biosynthesis from platelets, as reflected by excretion of DTxB₂, was significantly depressed after aspirin challenge. Inhibition of Tx synthesis began rapidly and was almost maximum at the 3–6- and 6–9-h periods. Urinary DTxB₂ did not return to baseline values even by the collection at 9–24 h. The pattern of changes of DTxB₂ with time was not different between the AIA and the ATA groups (Table 2). A slightly attenuated reduction of DTxB₂ excretion was detected in the AIA group, which may be reflected in the lower

Table 2. Percent changes or urinary LTE₄, 9 α ,11 β -PGF₂, methylhistamine, and DTxB₂ concentrations after provocation with aspirin

	ATA (n = 10)					AIA (n = 10)						
	Before	0	0-3	3-6	6-9	9-24 h	Before	0	0-3	3-6	6-9	9-24 h
LTE ₄	100	173 ± 61.6 (67.9 ± 53.0 pg/mg creatinine)	134.0 ± 66.1	156.0 ± 90.8	121.0 ± 54.3	148.0 ± 87.6	100	150.0 ± 140 (298.5 ± 388.7 pg/mg creatinine)	1814.0 ± 3475.4 [‡]	1413.0 ± 1344.4 [‡]	629.0 ± 567.4 [‡]	175.0 ± 103
9α,11β-PGF ₂	100	106 ± 57.8 (181.2 ± 197 pg/mg creatinine)	83.3 ± 51.8	89.0 ± 48.4	82.9 ± 41.2	107.0 ± 59.3	100	97.7 ± 4.0 (168.6 ± 232.6 pg/mg creatinine)	300.0 ± 287**	173.0 ± 81.8**	135.0 ± 103	116.0 ± 57.8
Methylhistamine	100	88.3 ± 8.7 (214.3 ± 119.8 ng/mg creatinine)	99.9 ± 14.8	98.7 ± 18.8	115.1 ± 21.1*	119.2 ± 25.0	100	99.2 ± 10 (136.6 ± 42.7 ng/mg creatinine)	171.0 ± 64.2**	145.0 ± 54.4**	153.0 ± 51.2**	137.0 ± 54.3
DTxB ₂	100	89.1 ± 25.6 (1340 ± 987.6 pg/mg creatinine)	47.6 ± 27.1**	30.7 ± 5.3 [‡]	34.9 ± 13.4 [‡]	41.4 ± 19.0 [‡]	100	103.0 ± 6.8 (1014 ± 748 pg/mg creatinine)	83.7 ± 54.1	50.0 ± 24.0 [‡]	42.4 ± 23.4 [‡]	52.4 ± 26.4 [‡]

Significantly different from baseline concentration, * $P < 0.02$, ** $P < 0.01$, [‡] $P < 0.001$. Figures in parenthesis show basal concentration of each compound.

dosage of aspirin in that group. When urine was collected from three normal subjects who received placebo over a similar time period, the concentration of DTxB₂ during the first hours after awakening and the 9-24-h period was significantly higher than that observed during the 0-3-, 3-6-, and 6-9-h periods (data not shown). As similar observations of diurnal variation in DTxB₂ excretion have already been reported (36), it cannot be ignored that diurnal variation may contribute to the data of DTxB₂ excretion and may complicate the recognition of the true real inhibition pattern.

When sensitivity to aspirin was defined as the interpolated concentrations of aspirin provoking a 20% reduction in FEV_{1.0} (PD₂₀, mg/ml), PD₂₀ values did not correlate with either the absolute concentration or the percent increase of LTE₄ concentration in AIA. The number of patients documented may be too small to allow us to correlate peptidyl-LT production with clinical symptoms.

When increases in the concentrations of all the urinary metabolites were expressed in terms of the maximum percentage increases at the peak response, the percentage increases in LTE₄, 9 α ,11 β -PGF₂, and methylhistamine were significantly higher in the AIA group (Fig. 1).

The patient who developed asthmatic symptoms after accidental percutaneous application of both flurbiprofen and indomethacin excreted higher concentrations of LTE₄ (6850 pg/mg creatinine) and 9 α ,11 β -PGF₂ (292 pg/mg creatinine) on the day of onset than during the following 4 consecutive days (LTE₄, 384, 189, 355, and 315 pg/mg creatinine; 9 α ,11 β -PGF₂, 167, 123, 151, and 123 pg/mg creatinine).

Discussion

Evidence that LTs play a role in the induction of aspirin sensitivity has been demonstrated by the findings that AIA patients excreted significantly more urinary LTE₄ than ATA patients (3-6). Cowburn et al. have recently reported that LTE₄ concentration in bronchoalveolar lavage fluid was also significantly higher in AIA patients (12), although some workers failed to observe differences in basal LTE₄ concentration in bronchoalveolar lavage fluid between the two groups (37, 38). We found that the AIA group exhibited significantly higher urinary LTE₄ concentrations than the ATA group, even during periods of clinical stability. In contrast, the baseline concentrations of urinary 9 α ,11 β -PGF₂, another metabolite presumably related to mast-cell activation, were not significantly different between the two groups. O'Sullivan et al. also reported that the failure to detect increased levels of urinary 9 α ,11 β -PGF₂ at the baseline concentrations in AIA patients, despite the fact that high levels of LTE₄ can be detected (35), suggests that the cellular source of augmented excretion of LTE₄

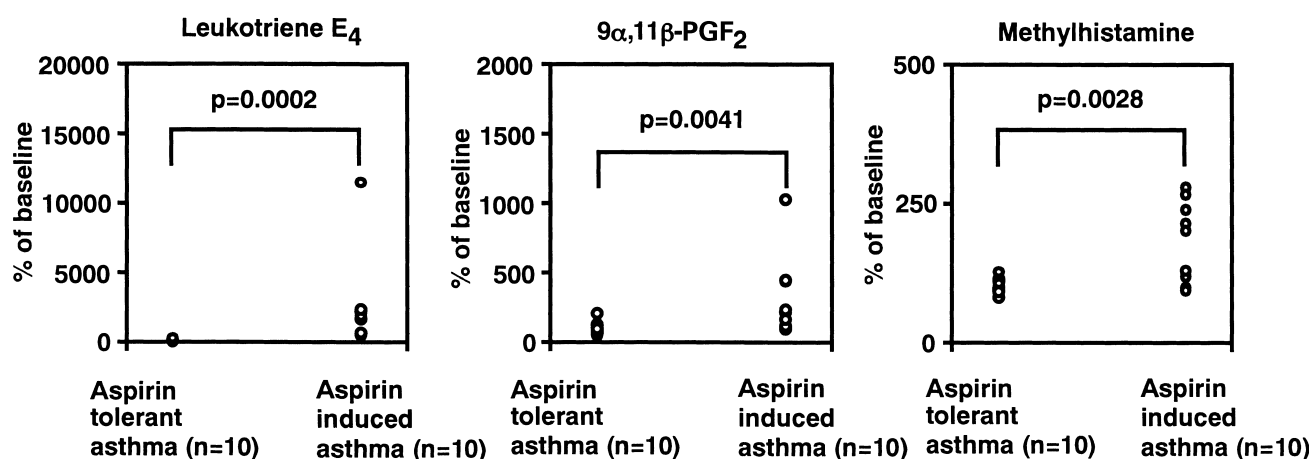


Figure 1. Percent increases of urinary LTE₄, 9α,11β-PGF₂, and methylhistamine concentrations after provocation with aspirin.

at the baseline levels and increased excretion of LTE₄ after aspirin provocation may be different. Thus, we could not decide whether increased excretion of urinary LTE₄ at the baseline levels may reflect ongoing activation of mast cells, or may be attributed to cells other than mast cells. Our previous observations suggested that two patients, intubated for severe spontaneous asthma attacks, excreted LTE₄ at concentrations of around 300 pg/mg creatinine (data not shown). In this respect, it is of great interest that the basal excretion of LTE₄ concentrations in the AIA group was almost identical to that observed in the intubated patients.

Significant increases in LTE₄ concentrations occurred in urine, bronchoalveolar fluid, and nasal lavage fluid after inhalation or instillation of aspirin in AIA patients, but not in ATA patients (3, 4, 7, 8, 12, 37, 39, 40). We have shown that intravenous injection of aspirin also induced a significantly higher excretion of both LTE₄ and 9α,11β-PGF₂ in the AIA group than the ATA group. A major finding of this study is that intravenous injection of aspirin caused a slight but statistically significant increase of methylhistamine in the urine of AIA patients, because there are no previous reports of enhanced excretion of urinary methylhistamine in AIA patients after provocation of aspirin. This is probably related to the findings that intravenous administration of aspirin induced remarkably higher increases of urinary concentration of LTE₄ (a geometric mean 13.1-fold) and 9α,11β-PGF₂ (2.39-fold) than the extent of increase described by oral or inhaled provocation of aspirin. In addition, higher excretion of LTE₄ (18-fold) and 9α,11β-PGF₂ (twofold) was also observed in a patient who developed asthmatic symptoms after topical application of nonsteroidal anti-inflammatory drugs. This increase in urinary metabolites of mast-cell-associated mediators provides a possible link between mast-cell activation and bronchoconstriction in AIA patients. It has already been assumed that histamine may be released during the

onset of aspirin sensitivity because an antagonist to the histamine H₁-receptor protects the reactions (41). It is unlikely that other inflammatory cells possessing a capacity to generate both LTC₄ and PGD₂, such as eosinophils, may also be responsible for the production of some of these mediators, as eosinophils generate much smaller amounts of PGD₂ than mast cells (42–44); furthermore, eosinophils do not release histamine. However, it seems possible that eosinophils may be partially responsible for the increased excretion of LTE₄ after aspirin challenge, because it has been reported that indomethacin elevated LTC₄ generation by eosinophils (45). Thus, we could not exclude the possibility that histamine was released as a result of activation of eosinophils because eosinophil granule major basic protein has been shown to degranulate human basophils, causing noncytotoxic histamine release (46). Although the possibility that mast cells may be involved in the pathogenesis of aspirin-induced asthma is also supported by a number of studies (15, 16, 44), it is uncertain whether aspirin itself stimulates mast cells or influences ongoing mast-cell activation by decreasing regulatory prostaglandins through the cyclooxygenase inhibition or, less likely, through the shunting effect which diverts arachidonates into lipoxygenase pathways, because we have not observed shunting of arachidonic acid in a single-cell suspension of purified human mast cells (47). In regard to the increase of 9α,11β-PGF₂, our results confirm the recent findings of O'Sullivan et al. (35), but conflict with other reports that concentrations of PGD₂ did not increase in bronchoalveolar fluid or nasal lavage fluid after inhalation of aspirin or instillation of aspirin in the bronchus (37–39). When chopped tissue or dispersed cells of polyps resected from AIA patients were incubated with aspirin *in vitro*, we could not detect histamine release and PGD₂ generation from the mast cells (data not shown), suggesting that degranulation of mast cells in response to aspirin occurred exclusively *in vivo*.

Aspirin suppressed the urinary level of DTxB₂, suggesting that aspirin at least adequately inhibited platelet cyclooxygenase. There is an apparent paradox here, in that aspirin failed to diminish the excretion of 9 α ,11 β -PGF₂ or induce an increase in synthesis of PGD₂ in the AIA group. We do not have a satisfactory explanation for these findings. However, it appears reasonable to consider that the dose of aspirin may not have caused significant inhibition of the cyclooxygenase in mast cells, because it is known that the platelet cyclooxygenase is uniquely sensitive to inactivation by aspirin (32, 48). In contrast to the results obtained after administration of aspirin, attenuated excretion of urinary DTxB₂ did not occur on the day of onset in the patient who suffered an asthmatic attack after percutaneous administration of nonsteroidal anti-inflammatory drugs. There are several possible explanations of this difference. First, unlike aspirin, indomethacin does not possess preferential sensitivity to platelet cyclooxygenase (32, 49). Secondly, platelets lack nuclei and are incapable of

protein synthesis. Thus, the effect of aspirin on platelets is permanent and persists for the life of the platelet (49). In contrast, most other nonsteroidal anti-inflammatory drugs do not result in irreversible inactivation of cyclooxygenase, and thus the duration of the cyclooxygenase inhibition is shorter than that caused by aspirin (36).

Sanak and colleagues have recently reported a polymorphism that may result in a new consensus binding site for the AP2 transcription factor, and thus may increase transcription of LTC₄ synthase, at the position 444 upstream of the start codon in LTC₄ synthase (13, 14). Relation between the transversion and augmented translation levels is still unclear, as there have been conflicting reports even in transfection experiments (14, 50, 51).

In conclusion, these findings provide indirect evidence for the involvement of mast-cell activation, after intravenous administration of aspirin, in ATA, although the precise mechanism of mast-cell activation so far remains unknown.

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