

Association of urinary leukotriene E₄ excretion during aspirin challenges with severity of respiratory responses

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Background: Asthmatics with aspirin- (ASA) sensitive respiratory disease (ASRD) have a spectrum of respiratory reactions during oral ASA challenge that vary in severity and are temporally associated with leukotriene (LT) formation.

Objective: This study investigates the relationship of the severity of ASA-induced respiratory reactions to urinary LTE₄ excretion.

Methods: Asthmatics with suspected ASRD underwent oral ASA challenges. Urinary LTE₄ levels were measured at baseline, during the reaction, and after acute ASA desensitization. **Results:** Asthmatics who had respiratory reactions during oral ASA challenges were divided into 3 groups: asthmatics with naso-ocular reactions and <15% decline from baseline FEV₁ values (group 1), asthmatics with a decline in FEV₁ of 20% to 30% (group 2), and asthmatics with a decline in FEV₁ of >30% (group 3). There were no significant differences in age, baseline FEV₁ values, use of inhaled corticosteroids, daily prednisone doses, prednisone bursts, duration of reactions, or average provoking doses of ASA among the groups. At baseline group 3 asthmatics had significantly higher urinary LTE₄ levels than those in groups 1 or 2. At the time of respiratory reaction to ASA, the urinary LTE₄ levels rose significantly in all groups but were significantly greater among group 3 asthmatics compared with those in groups 1 and 2.

Conclusion: The severity of the respiratory reactions during oral ASA challenges was associated with the degree of elevation of baseline LTE₄ synthesis. Our results suggest that asthmatics with ASRD have a spectrum of respiratory tract reactions in which leukotrienes play a distinguishing role. (J Allergy Clin Immunol 1999;104:559-64.)

Key words: Aspirin-sensitive asthma, aspirin challenge, peptidoleukotrienes, LTE₄

After ingestion of aspirin (ASA) or nonsteroidal anti-inflammatory drugs (NSAIDs), asthmatic patients with

Abbreviations used

AA:	Arachidonic acid
ASA:	Aspirin
ASRD:	Aspirin-sensitive respiratory disease
COX:	Cyclo-oxygenase
GCRC:	General Clinical Research Center
5-LO:	5-Lipoxygenase
LT:	Leukotriene
NSAID:	Nonsteroidal anti-inflammatory drugs
PG:	Prostaglandins
Tx:	Thromboxane

ASA-sensitive respiratory disease (ASRD) have attacks of asthma that are usually accompanied by naso-ocular signs and symptoms of nasal congestion, mucous secretion, and tearing.^{1,2} Particularly when challenged with small doses of ASA, some patients have only a naso-ocular reaction, without associated bronchospasm.^{3,4} ASRD affects 10% to 20% of all asthmatic patients and up to 40% of asthmatic patients with nasal polyps and sinusitis.² A growing body of evidence implicates inhibition of cyclo-oxygenase (COX) in unidentified cells in the respiratory tract as an essential initiating event leading to ASA-induced respiratory reactions.^{5,6} Asthmatic patients with ASRD can be desensitized to ASA after repeated exposure to increasing doses of ASA.^{4,7} Treatment of ASA-desensitized asthmatic patients with 650 mg of ASA given twice daily led to clinical improvement in the majority of patients with ASRD and to reduced requirements for systemic corticosteroids.^{8,9}

Various metabolites of arachidonic acid (AA) play an active role in airway reactivity and inflammation.^{6,10} Thromboxanes (Tx) and prostaglandins (PG) are generated by the COX pathway, yielding TxB₂ and the bronchodilators PGI₂ and PGE₂. PGE₂ has been shown to inhibit 5-lipoxygenase (5-LO)¹¹⁻¹³ to down-regulate the release of mast cell products¹⁴ and to inhibit eosinophil degranulation.¹⁵ Products of 5-LO include the chemoattractant leukotriene B₄ (LTB₄) and the potent bronchoconstrictors LTC₄ and LTD₄. They are rapidly metabolized to the more stable form LTE₄, which is excreted in the urine, therefore reflecting total-body sulfidopeptide LT production.

Christie et al¹⁶ reported that LTE₄ concentrations in the urine were elevated at baseline in ASA-sensitive asthmatic patients before oral ASA challenge. During oral ASA challenges urinary LTE₄ levels increased substan-

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tially, coinciding with ASA-induced respiratory reactions. The rise in urinary LTE_4 levels was followed by a return to prechallenge levels 4 to 6 hours after reactions. These observations have been confirmed independently in other centers.^{17,18} The appearance of LTs during ASA-induced respiratory reactions and their modulation by ASA ingestion strongly implicate lipoxygenase products as responsible for stimulating lower, and perhaps, upper respiratory tract reactions in patients with ASRD.

We hypothesized that asthmatic patients with ASRD display heterogeneous features that are manifestations of the underlying abnormalities in the 5-LO pathway and that are specific target organ responses to these mediators. The purpose of our study was to examine the association of urinary LT excretion with the severity of the respiratory response during oral ASA challenges. A second aim of the study was to contrast clinical characteristics, time to onset of the reaction, provoking dosages of ASA, and duration of the respiratory reactions among asthmatic patients grouped according to the severity of their respiratory responses to ASA.

METHODS

Aspirin challenges

Asthmatic patients over the age of 18 years who were suspected of having ASA-sensitive asthma were admitted to the General Clinical Research Center (GCRC) at Scripps Clinic between 1993 and 1998 and signed institutional consent forms for human subject experimentation. None were receiving LT modifier drugs. For most patients, the individualized program of controller medications, including inhaled and systemic corticosteroids or theophylline, were continued throughout the challenges. β -Agonists, antihistamines, cromolyn sodium, and nedocromil sodium were withheld at the time of admission to the GCRC, resulting in withdrawal of these medications for 72 hours before ASA challenges. Asthma was considered stable if FEV_1 values were $>70\%$ predicted at baseline and varied by less than 15% during 9 hours of placebo challenges on days 2 and 3.⁷ FEV_1 values of some asthmatic patients remained less than 70% predicted despite the addition of systemic corticosteroids. These patients were either excluded from further study or discharged and readmitted after receiving bursts of corticosteroids.

Once airway stability was achieved, subjects underwent placebo-controlled, single-blind graded oral ASA challenges. Serial FEV_1 values were obtained at least hourly between 6 AM and 7 PM and at the time of any symptoms during the day of challenge. Oral ASA challenges were conducted with the following schedule. The patient was admitted on day 1 and placebo challenges were conducted on days 2 and 3. Beginning on day 4, single-blind ASA challenges were administered as follows: at 7 AM, 30 mg of ASA; at 10 AM, 60 mg of ASA; and at 1 PM, 100 mg of ASA. If no reaction occurred on day 4, ASA challenges were resumed on day 5 as follows: at 7 AM, 150 mg of ASA; at 10 AM, 325 mg of ASA; and at 1 PM, 650 mg of ASA. The evidence for an ASA-induced respiratory reaction was assessed at least hourly and consisted of the following: naso-ocular reactions (the presence of rhinorrhea, nasal congestion, ocular injection, periorbital edema, flushing) or bronchospasm. If a respiratory reaction occurred after a challenge dose of ASA, challenges were suspended until resolution of naso-ocular signs and symptoms or bronchospasm. Nebulized β_2 -agonist were used to treat ASA-induced asthmatic reactions. Topical oxymetazoline was used to treat nasal obstruction, and topical ocular antihistamines were used to treat ocular reactions.

Incremental ASA desensitization was conducted beginning at 7 AM on the day after resolution of ASA-induced respiratory reactions. The provoking dose of ASA from the prior day's challenge was repeated and, if tolerated, incremental escalation of the ASA doses to 650 mg was undertaken with 3 hours between each dose. If repetition of the provoking dose of ASA elicited respiratory symptoms, desensitization was suspended until resolution of respiratory signs and symptoms. The provoking dose of ASA was repeated until tolerated and then the ASA dose was escalated as defined above. Acute desensitization was completed within 1 to 3 days after the first respiratory reaction in all patients.

We also recorded various parameters associated with the respiratory reaction. These included "time to onset," which was the elapsed time from ingestion of ASA to the first detection of respiratory reaction, and the "reaction duration," which was the amount of time from the onset of detectable respiratory reactions until all signs and symptoms of the reaction had disappeared.

Patient selection

All subjects who underwent graded oral ASA challenges met the criteria for a diagnosis of asthma as outlined by the American Thoracic Society. Each of the subjects had a convincing history of significant reactions to inadvertent ingestion of 650 mg of ASA in the past with classic features of naso-ocular symptoms and severe bronchospasm. After such accidental ASA ingestion, marked and protracted asthmatic reactions dominated the clinical picture, and emergency department treatment or hospitalization was usually required. After stabilization of the asthma, the patients were referred to us for graded oral ASA challenges followed by ASA desensitization.

Consecutive patients with positive ASA challenges were included and all subjects had urine samples obtained at all 3 time points. The asthmatic patients were divided into 3 groups entirely on the basis of the decline of FEV_1 values from baseline during the reaction to oral ASA challenges. Group 1 consisted of 21 asthmatic patients whose FEV_1 values declined $\leq 15\%$ from baseline values (same as placebo range) but who had clear-cut naso-ocular reactions. Twenty-eight asthmatic patients whose FEV_1 values declined from 20% to 30% of baseline values comprised group 2. Twenty-five asthmatic patients with declines in FEV_1 values $>30\%$ from baseline values were assigned to group 3. Fourteen patients whose FEV_1 values declined between 15% to 20% from baseline values were excluded from this particular study because they did not represent a clearly distinct group.

Measurement of LTE_4

Reagents. 4-Hydroxy-tempo (4-hydroxytetramethyl-piperidinoxy-free radical) was purchased from Sigma (St Louis, Mo). C18 Sep-Pak cartridges were supplied by Waters Associates (Milford, Mass). LTE_4 enzyme immunoassay kits were obtained from Cayman Chemical (Ann Arbor, Mich).

Procedure. "Spot" urine samples were obtained before oral ASA challenges (baseline) were begun, within minutes of documented reaction to oral ASA challenges (reaction), and 3 hours after tolerating 650 mg of ASA (acute desensitization). Urine collection and measurement of LTE_4 followed a modification of the procedure described by Manning et al.¹⁹ Urine was collected and centrifuged, and 10-mL aliquots of the supernatant were added to 15-mL polypropylene conical tubes containing 450 μL of the oxygen radical scavenger 4-hydroxy-tempo (0.1 mol/L) and sodium hydroxide (10 mol/L) to achieve a final pH >9 . The samples were immediately frozen and stored at -70°C until the assay was performed. A second aliquot (1 mL) was analyzed for creatinine levels. On thawing, the pH was adjusted to 5.4 with acetic acid and samples were spun at 8000g for 10 minutes at 4°C . C18 Sep-Pak cartridges were pre-

TABLE I. Baseline characteristics of ASA-sensitive asthmatic study subjects

Group	Size (n)	Age (y)	Inhaled steroid/day (μg)	Prednisone dose/day (mg)	No. of bursts in prior year
1	21	47.5 ± 2.8	620.0 ± 119.6	4.2 ± 2.0	0.76 ± 0.45
2	28	48.5 ± 2.2	632.9 ± 159.0	2.4 ± 0.9	1.54 ± 0.7
3	25	44.3 ± 2.6	528.0 ± 122.1	5.7 ± 1.7	1.96 ± 0.58

Summary statistics are given as mean ± SE for asthmatic patients with decline in FEV₁ values of <15% but with prominent naso-ocular reactions (group 1), asthmatic patients with decline in FEV₁ values of 20% to 30% (group 2), and asthmatic patients with decline in FEV₁ values of >30% (group 3).

TABLE II. Characteristics of ASA-induced respiratory reactions

Group	Drop in FEV ₁ (%)	Time to onset (h)	Reaction duration (h)	ASA dose (mg)	Naso-ocular reactions	Laryngeal reactions
1	4.3 ± 1.1	1.7 ± 0.1	4.9 ± 0.7	66.4 ± 5.3	21/21	0/21
2	24.1 ± 0.6	2.1 ± 0.1	5.0 ± 0.4	66.6 ± 4.7	26/28	2/28
3	43.8 ± 1.8	1.7 ± 0.1	6.5 ± 0.9	60.0 ± 4.0	24/25	2/25

Summary statistics are given as mean ± SE for asthmatic patients in groups 1, 2, and 3.

washed with distilled water (10 mL), methanol (10 mL), and 0.5% EDTA buffer, pH 5.4 (20 mL). Ten milliliters of urine was applied to the Sep-Pak, washed with 20 mL of distilled water, and the LTs were eluted with 4 mL of methanol. The methanol fraction was dried under a nitrogen stream and reconstituted in 400 μL of 50% aqueous methanol. LTE₄ was measured in triplicate by enzyme immunoassay on all samples according to the manufacturers' instructions and quantified with use of a Titertek Multiscan MCC/340 ELISA reader. LTE₄ levels are expressed as picograms per milligram of creatinine.

Statistical analysis

Multivariate analysis of variance was used to compare LTE₄ values in the 3 groups of subjects under conditions of baseline, reaction, and desensitization. Analyses were performed on log-transformed LTE₄ values because the log transformation induced approximate normality in these values, correcting for skewness. Significant group differences were found with Wilks' lambda (the likelihood ratio criterion); individual intergroup and intragroup comparisons were then made by specifying particular general linear hypotheses, with the resulting F tests reported here. *P* values are nominal and are not corrected for the multiplicity of tests performed. Pearson correlation coefficients were used to determine the association between various LTE₄ levels or between LTE₄ levels and FEV₁ values. Calculations were performed in Instat 6.0 for Windows (Intuitive Co. for Researchers, San Diego, Calif). Summary statistics are reported as means ± SEs.

RESULTS

Characterization of patients

Table I depicts the baseline characteristics of asthmatic patients with a decline in FEV₁ values of <15% but with prominent naso-ocular reactions (group 1), asthmatic patients with a decline in FEV₁ values of 20% to 30% (group 2), and asthmatic patients with a decline in FEV₁ values of >30% (group 3), respectively. Many patients with ASRD were not receiving daily oral glucocorticoids but required corticosteroid bursts during the year preceding oral ASA challenges. Bursts of corticosteroids were defined as a daily dose of 30 to 60 mg of prednisone (or equivalent methylprednisolone) with tapering to 0 mg

over the subsequent 14 to 21 days. There were no significant differences in the ages of the asthmatic patients, use of inhaled corticosteroids among subjects, maintenance prednisone doses, or number of corticosteroid bursts during the previous year among the 3 groups (*P* > .05 for all comparisons, 2-tailed *P* value).

Table II depicts the characteristics of the asthmatic patients during ASA-induced respiratory reactions. During reactions to ASA challenges there were no significant differences among the asthmatic patients in the 3 groups with respect to the duration of the reactions or in the average provoking doses of ASA (*P* > .05 for both comparisons, 2-tailed *P* values). The onset of symptoms for asthmatic patients in group 1 occurred significantly earlier than the onset of symptoms for asthmatic patients in group 2 (*P* = .04, 2-tailed *P* values). This response occurred despite the presence of naso-ocular signs and symptoms in all but 2 of the 28 asthmatic patients in group 2. There was an insignificant trend toward an earlier onset of symptoms in patients whose FEV₁ values declined >30% from baseline compared with those whose FEV₁ values declined 20% to 30% from baseline (*P* = .10, 2-tailed *P* values). By contrast, the difference in time of onset of respiratory symptoms did not differ among asthmatic patients in groups 1 and 3 (*P* > .05, 2-tailed *P* values).

Comparisons of the decline in FEV₁ values among the 3 groups revealed highly significant differences (*P* < .0001 for group 1 vs group 2, group 1 vs group 3, and group 2 vs group 3). A majority of asthmatic patients in groups 2 and 3 had prominent naso-ocular reactions in addition to bronchospasm. A few patients with naso-ocular and asthmatic reactions to oral ASA challenges manifested prominent inspiratory crowing sounds over the upper trachea and neck. These characteristic physical findings were accompanied by a flat or notched inspiratory loop in the flow-volume curve recorded during the time of the respiratory reactions. These asthmatics were designated as also having laryngeal reactions to oral ASA challenges, and this finding was identified separately.

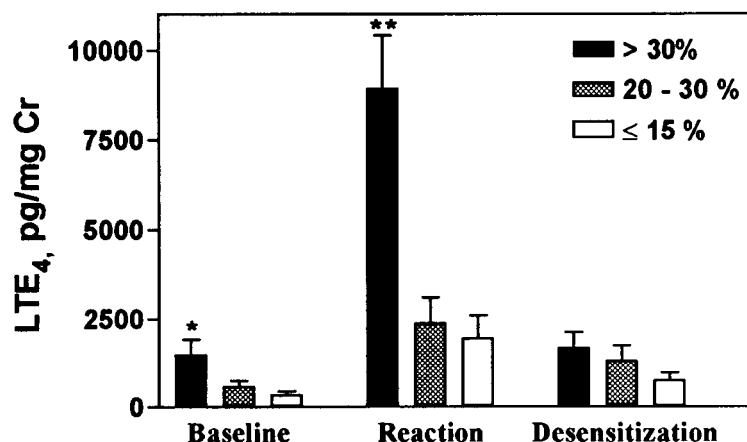


FIG 1. Urine specimens were collected at indicated time points and LTE₄ levels were assayed and expressed in terms of picograms per milligram of creatinine (Cr). Urinary LTE₄ levels were grouped according to decline from baseline FEV₁ values during respiratory reactions to oral ASA challenges. Values for each group represent means $\pm$ SE; for group 1 (n = 21), group 2 (n = 28), and group 3 (n = 25). Group 3 urinary LTE₄ levels were significantly greater than levels in groups 1 and 2 at baseline (asterisk, $F_{2,71} = 6.162$, $P = .004$) and during ASA-induced respiratory reactions (two asterisks, $F_{2,71} = 16.237$, $P < .001$) but not at acute desensitization ($F_{2,71} = 1.633$, $P = .203$).

Urinary LTE₄ levels during ASA challenge

Fig 1 depicts urinary LTE₄ levels during the course of ASA challenges in all ASA reactors grouped according to type and severity of reactions. There were significant differences overall among the 3 groups in LTE₄ values from the multivariate analysis of variance (Wilks' likelihood ratio test, $F_{6,138} = 5.75$, $P < .001$). Subsequent univariate F tests revealed that the 3 groups significantly differed at baseline ($F_{2,71} = 6.162$, $P = .004$), with group 3 having significantly larger LTE₄ values than the other 2 groups.

At the time of respiratory reactions to ASA, univariate F tests again revealed significant differences among the 3 groups ($F_{2,71} = 16.237$, $P < .001$) with LTE₄ levels in group 3 (8960 pg/mg creatinine) being significantly greater than levels in either group 1 (1944 pg/mg creatinine) or group 2 (2400 pg/mg creatinine). Importantly, the asthmatic patients in group 3, who had the greatest decline in FEV₁ values (>30%), had markedly elevated levels of mean urinary LTE₄ during their reactions to ASA compared with baseline levels (1492 vs 8960 pg/mg creatinine). Interestingly, for group 3 the relative increase in mean LTE₄ levels was 6.0-fold compared with 5.3-fold for group 1 and 4.1-fold for group 2.

Within-group comparisons of LTE₄ levels revealed qualitatively similar patterns of response across the 3 groups. LTE₄ values at reaction were significantly higher than values at baseline ($F_{2,71} = 25.183$, $P < .001$ for group 1; $F_{2,71} = 21.197$, $P < .001$ for group 2; and $F_{2,71} = 72.204$, $P < .001$ for group 3). Similarly, LTE₄ values at reaction were significantly higher than values at desensitization ($F_{2,71} = 8.140$, $P = .006$ for group 1; $F_{2,71} = 5.685$, $P = .020$ for group 2; and $F_{2,71} = 59.709$, $P < .001$ for group 3).

Urinary LTE₄ levels declined after achieving acute desensitization to ASA in all 3 groups. Among the 3 groups, no statistically significant differences in urinary LTE₄ levels existed at the time of acute desensitization (group 1, 752 pg/mg creatinine; group 2, 1314 pg/mg creatinine; and group 3, 1683 pg/mg creatinine; $F_{2,71} = 1.633$, $P = .203$). Furthermore, LTE₄ levels at baseline and after desensitization were of comparable magnitude and were statistically different only for group 2 ($F_{2,71} = 3.879$, $P = .053$ for group 1; $F_{2,71} = 4.210$, $P = .044$ for group 2; and $F_{2,71} = 0.087$, $P = .77$ for group 3).

DISCUSSION

Prior reports of oral aspirin challenges have demonstrated baseline levels of urinary LTs in ASA-sensitive asthmatic patients that were elevated compared with those of non-ASA-sensitive asthmatic patients.^{16-18,20} Coincident with the onset of respiratory reactions to oral ASA challenges, LTE₄ levels rose abruptly and declined to baseline levels within 4 hours of the ASA-induced respiratory reactions in these studies. Because non-ASA-sensitive control asthmatic patients, when challenged with ASA, were not found to have increased levels of urinary LTE₄, these biochemical changes were reported to be unique to ASA-sensitive asthmatic patients.¹⁶⁻¹⁸ Our study, which did not include ASA-tolerant control asthmatic subjects, nevertheless confirms the pattern of elevated baseline urinary LTE₄ values in ASA-sensitive asthmatic patients. As was the case in prior studies,¹⁶⁻¹⁸ LTE₄ urine levels increased further with the onset of ASA-induced respiratory reactions (Fig 1).

After initial respiratory reactions to ASA, our patients with ASRD immediately underwent acute desensitization to ASA followed by long-term treatment with daily ASA.

The desensitization procedure allowed us to measure urinary LTE_4 levels after ingestion of a uniform dose of 650 mg of ASA taken at the completion of ASA desensitization. Urinary LTE_4 levels after acute desensitization were near baseline levels (Fig 1), a decline similar to the decline that occurred within 4 hours after ASA-induced reactions in prior studies.^{16-18,20} The mechanisms explaining ASA desensitization are incompletely understood. However, interruption of LT synthesis did not occur; rather the formation of LTs was reset at about the same rate as baseline formation. This occurred despite removal of the inhibitory effects of PGE_2 on 5-LO.¹¹⁻¹³ Therefore additional explanations for the clinical benefit of ASA desensitization, other than effects on LT synthesis, will be required.

Our study also shows that the extent and severity of the respiratory reactions to aspirin are positively associated with variously increased LTE_4 levels in the urine. Baseline synthesis of LTE_4 in our 3 groups was high compared with low levels of LTE_4 found in normal subjects and ASA-tolerant asthmatic patients in prior studies¹⁶⁻¹⁸ and in our own laboratory (study in currently in preparation for a future publication). Among each of our 3 asthmatic groups in the current study, variably increased levels of LTE_4 values probably reflect the degree of underlying airway reactivity at the times of oral ASA challenges. In turn, elevated baseline LT formation in asthmatics with ASRD appeared to coincide with the severity of the respiratory response to oral ASA challenge. Thus severe bronchospasm and very high rates of LT synthesis during respiratory reactions followed the remarkably elevated baseline LTE_4 levels in group 3 asthmatic patients. In comparison, the lower baseline levels of LTE_4 in groups 1 and 2 resulted in modest increases in LT formation accompanied by only naso-ocular or mild bronchospasm during recorded reactions to ASA. All 3 groups had similar 4- to 6-fold increases in LTE_4 excretion with the onset of respiratory reactions to ASA. However, a 6-fold increase from a high baseline synthesis produced the highest "reaction" level of LTs. Thus the underlying rate of baseline LT synthesis appeared to be the controlling variable predicting the severity of ASA-induced respiratory reactions.

On the other hand, basal LT synthesis did not predict the occurrence of symptoms limited to the upper respiratory tract (group 1) versus the upper and lower respiratory tracts (groups 2 and 3). Because all our patients gave histories of upper and lower airway responses to inadvertent ingestion of 650 mg of ASA, we can only speculate that the upper and lower airways may have differing sensitivities to the effects of LTs. It would not be ethical to challenge suspected ASA-sensitive asthmatic patients with an initial dose of 650 mg of ASA, and therefore this speculation cannot be tested in the laboratory. Nevertheless, it is likely that individual variability in response to oral ASA challenges was determined by the extent of underlying inflammation, as reflected in baseline LT formation, by the provoking dose of ASA, and by differing sensitivities of the upper or lower respiratory tracts to the effects of ASA.

ASA-induced reactions are clearly dose dependent and vary from time to time within an individual, depending on the degree of bronchial hyperactivity at the time of challenge.² A surprising finding in this study was the lack of differences in the provoking doses of ASA that initiated the respiratory reactions in our 3 study groups. Equivalent provoking doses of ASA induced upper and lower respiratory reactions that differed markedly in severity among the 3 groups. Therefore the severity of respiratory reactions may be determined by individual variability in LT formation as well as by advancing doses of ASA. This individual responsiveness to challenge with ASA appeared to be linked most closely to baseline rates of LT synthesis at the time of ASA ingestion. Thus both ASA provoking doses and individual responses to those advancing doses appeared to govern LT synthesis.

One hypothesis about the mechanism of ASRD argues for increased sensitivity of the airways to LTs that also markedly diminishes after ASA desensitization.²¹⁻²³ Alternatively, a second hypothesis postulates that COX blockade by ASA shunts AA into the 5-LO pathway, resulting in increased formation of the potent bronchoconstrictors LTC_4 , LTD_4 , and LTE_4 .⁶ The effect of shunting is further magnified by the reduced synthesis of PGE_2 , which removes the potent inhibitory effects of PGE_2 on 5-LO¹¹⁻¹³ and on eosinophil and mast cell activation.^{14,15} Recently, enhanced expression of the COX-2 isozyme and of LTC_4 synthase in mast cells and eosinophils in the bronchial mucosa of patients with ASRD has been shown.²⁴⁻²⁶ These abnormalities in expression of critical enzymes in the metabolism of arachidonate imply that genetic polymorphisms in patients with ASRD may provide the biomolecular framework for the unique sensitivity to ASA in this subset of asthmatic patients.

Our results argue against the shunting of AA metabolites into the lipoxygenase pathway as the sole explanation for ASA-induced respiratory reactions. Shunting of AA metabolites may contribute during the initial phase of respiratory reactions to ASA challenges. However, shunting could not continue as a dominant force because urinary LTE_4 levels fell during desensitization despite continued COX blockade. The increased baseline secretion of products from both the COX and 5-LO pathways in patients with ASRD supports a genetic defect. Such a defect would require enhanced expression of both a peculiar ASA-sensitive COX-1 or COX-2 isozyme and of an up-regulated LTC_4 synthase or of enhanced expression of phospholipase A_2 in mast cells and eosinophils in the bronchial mucosa.

Direct stimulation by ASA/NSAIDs of resident mast cells and eosinophils in the respiratory tract probably occurs. Such stimulation would explain both the release of histamine, tryptase, and eosinophil cationic protein and the triggering of increased AA metabolism and of 5-LO production.^{27,28} Whatever the ultimate enzyme dysregulation underlying ASRD is determined to be, ASA enhances the synthesis of LTs to varying degrees. Our study demonstrates that this pathogenic step, underlying

the increased LT synthesis, ultimately determines the magnitude of subsequent lower respiratory reactions.

In summary, the spectrum of respiratory responses to oral ASA challenges appears to be heterogeneous. We have confirmed that LT formation is the essential component causing lower respiratory reactions to ASA. Individual reactions depend on the extent of underlying inflammation, as reflected by basal LTE₄ excretion, on differing sensitivities of the upper or lower respiratory tract to the effects of ASA, and on the provoking dose of ASA. Furthermore, we report the novel observations that LTs play some role, along with histamine, in the upper respiratory tract reactions induced by ASA ingestion and that individual asthmatic patients respond to the same ASA doses with varying degrees of LT formation. This association between elevation in urinary LTE₄ levels and severity of respiratory responses continues to support a critical, quantitative role for sulfidopeptide LTs in the pathogenesis of ASA-induced bronchoconstriction.

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