

Inhibition of monocyte leukotriene B₄ production after aspirin desensitization

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Aspirin-sensitive patients may be desensitized through a graded series of exposures to aspirin. We investigated the underlying mechanism of aspirin desensitization by measuring the release of leukotrienes B₄ and C₄ from calcium ionophore-stimulated peripheral blood monocytes. Compared with monocytes from normal volunteers (n = 5), monocytes from patients with aspirin-sensitive asthma (n = 10) released increased amounts of thromboxane B₂ (1060 ± 245 pg/ml vs 456 ± 62 pg/ml), leukotriene B₄ (861 ± 139 pg/ml vs 341 ± 44 pg/ml), and leukotriene C₄ (147 ± 31 pg/ml vs 56 ± 6 pg/ml) at baseline. After aspirin desensitization, thromboxane B₂ release was almost completely suppressed in both groups. Leukotriene B₄ release was significantly decreased in the aspirin-sensitive group (484 ± 85 pg/ml) but not in the normal subject group (466 ± 55 pg/ml). The need for prednisone decreased significantly after patients were desensitized to aspirin (10.4 ± 2.2 mg/day to 1.6 ± 2.8 mg/day). These results demonstrate that desensitization to aspirin results in decreased monocyte leukotriene B₄ release. On the basis of the bronchospastic and inflammatory potential of leukotrienes, the decrease in leukotriene release may contribute to the clinical improvement seen after aspirin desensitization. (J ALLERGY CLIN IMMUNOL 1995;96:148-56.)

Key words: Aspirin-sensitive asthma, aspirin desensitization, monocytes, arachidonic acid metabolism, leukotrienes

Patients with aspirin-sensitive respiratory disease (ASRD) experience bronchospasm with or without naso-ocular reactions after ingestion of aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs).¹ These patients can be desensitized to NSAIDs by repeated controlled exposure to small doses of aspirin in the hospital. After aspirin desensitization, patients with ASRD may receive daily maintenance doses of aspirin, and they usually experience improvement in their upper or lower respiratory tract disease.¹⁻⁶

The underlying mechanism or mechanisms for aspirin sensitivity and the beneficial effects of aspirin desensitization in patients with ASRD are

Abbreviations used

ASRD:	Aspirin-sensitive respiratory disease
EIA:	Enzyme immunoassay
FEV ₁ :	Forced expiratory vol
LT:	Leukotriene
NSAIDs:	Nonsteroidal antiinflammatory drugs
PL:	Phospholipase
Tx:	Thromboxane

not well understood. The potency of NSAIDs to provoke bronchospasm in patients with ASRD correlates with their potency to inhibit the cyclooxygenase enzyme.^{7,8} Furthermore, NSAIDs are structurally diverse, yet can "cross-desensitize" in that desensitization to one NSAID also desensitizes the patient to the other NSAIDs.^{9,10} These observations suggest that the products of arachidonic acid metabolism mediate the reactions to NSAIDs in patients with ASRD.

We recently reported that peripheral blood monocytes of patients with ASRD released elevated amounts of thromboxane (Tx) B₂ and leukotriene (LT) B₄ compared with normal mono-

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TABLE I. Clinical data before and after aspirin desensitization in patients with ASRD

Patient No.	Sex	Age (yr)	Baseline			Desensitization*		
			FEV ₁ (% predicted)	Prednisone (mg/day)	FEV ₁ (L)	Time (mo)	Prednisone (mg/day)	FEV ₁ (L)
1	F	44	74.6	10	2.15	10.5	0	2.67
2	M	49	71.4	30	2.51	10	0	2.74
3	F	41	85	4	2.21	5	4	NA
4	F	49	100	4	2.71	0.5	0	NA
5	M	32	85	0	3.62	2	0	3.66
6	F	29	79	10	2.24	5	0	NA
7	M	58	61	6	2.21	1	4	NA
8	F	54	80.5	30	2.11	6	0	1.96
9	F	56	69.6	10	1.76	4	7.5	1.84
10	M	21	54.5	0	2.28	3	0	2.24
Mean		43.3	76.1	10.4	2.38	4.7	1.6†	2.52‡
± SEM		5.1	4.5	2.2	0.03	1.7	2.8	0.12

NA, Not available.

*Aspirin 650 mg twice daily.

†*p* = 0.018 compared with baseline.

‡*p* = 0.295 compared with baseline (not significant).

cytes.¹¹ At the time of aspirin-induced bronchospasm, ASRD monocytes released less Tx_B₂ and more LTB₄ than at baseline. These patients were subsequently desensitized to aspirin, and we have now studied their monocytes at several time points after desensitization. We report that aspirin desensitization is associated with a significant decrease in the release of LTB₄, to levels that are indistinguishable from those of normal control subjects.

METHODS

Patient selection

Two groups of nonsmoking volunteer subjects, aged 21 to 58 years, were recruited for the study. All subjects gave informed consent, which was approved by the Human Research Committee of Scripps Clinic and Research Foundation. Patients with a history of asthmatic reactions to aspirin were admitted to the Clinical Research Center at Scripps Clinic to undergo oral aspirin challenge as previously described.¹² Clinical asthma and bronchial hyperreactivity were defined according to the American Thoracic Society guidelines of reversible airway obstruction, as demonstrated either by methacholine challenge or inhaled β_2 -agonist bronchodilation. Lung function tests with flow-volume measurements were performed on all patients. Serial forced expiratory volume in 1 second (FEV₁) values were obtained at least every hour between 6:00 AM and 9:00 PM during the entire study. Antihistamines, cromolyn sodium, and β_2 -agonists were discontinued at the time of admission (at least 24 hours before aspirin challenge) and were withheld throughout the hospitalization, except for nebulized β_2 -agonist treatments for

asthmatic reactions. Other regular medications were continued. Baseline blood samples were drawn on the day before aspirin challenge or immediately before ingestion of the first aspirin dose. Other blood samples were drawn at the indicated times.

Ten asthmatic subjects who were shown to have definite ASRD were investigated (Table I). Each of these patients experienced bronchospasm (fall in FEV₁ $\geq$ 20%) during oral aspirin challenge. Eight of these patients were receiving long-term oral treatment with prednisone (Table I). The second group consisted of five healthy nonasthmatic subjects (mean age, 34 $\pm$ 2 years) who were nonsmokers and who had not ingested NSAIDs for at least 4 weeks before the study. The healthy control subjects were not taking any medications.

Aspirin challenge

Aspirin challenges were performed in 10 patients with asthma as described by Stevenson.¹² After 1 day of single-blind placebo challenges to establish baseline FEV₁ values and airway stability, subjects were given increasing oral doses of aspirin, starting at 6 to 30 mg and increasing to 60, 100, 150, 325, and 650 mg. Challenges were continued at a minimum of 3-hour intervals with increasing doses until the subject reacted to aspirin. FEV₁ was measured at least every hour, and the patient's symptoms were monitored. A positive asthmatic response to aspirin was defined by a drop in FEV₁ of 20% or more. Reaction blood samples were obtained at the time of the first measurement of aspirin-provoked bronchospasm (drop in FEV₁ $\geq$ 20%) before administration of additional medication. In all cases, the reaction blood sample was obtained within 30 minutes after the onset of bronchospasm.

Aspirin desensitization

After the patient recovered from the aspirin reaction, the same provoking dose of aspirin was given repeatedly at intervals of 3 to 24 hours until reactions disappeared. Thereafter, increasing doses of aspirin were administered until the patient could ingest 650 mg of aspirin without adverse effect. Three hours after the patient first ingested 650 mg of aspirin, desensitization blood samples were drawn. The patients were then discharged while taking aspirin 650 mg twice a day, and the dose of prednisone was tapered as tolerated on an outpatient basis. Whenever possible, a repeat desensitization blood sample was obtained 3 hours after ingestion of 650 mg of aspirin after long-term treatment with aspirin for intervals determined by the ability of the subjects to return to the clinic for follow-up care.

Blood samples from healthy subjects were drawn before ingestion of 60 mg of aspirin, 3 hours later, 3 hours after ingestion of 650 mg of aspirin, and 3 hours after ingestion of aspirin after 650 mg of aspirin had been taken twice a day for 8 days.

Isolation of monocytes

Monocytes were isolated according to the method described by Boyum¹³ exactly as previously reported.¹¹ Leukocyte-rich plasma was prepared by dextran sedimentation of ethylenediaminetetraacetic acid plasma, and monocytes were isolated by density centrifugation (600 *g* for 10 minutes) on Nycodenz Monocytes (density = 1.068 gm/ml; Robbins Scientific, Sunnyvale, Calif.). Platelets were removed by centrifugation (50 *g* for 10 minutes) through autologous cell-free and platelet-free plasma. Monocytes were platelet-free and more than 95% pure as assessed by light microscopy. In some cases the absence of contaminating platelets was confirmed by fluorescence-activated cell sorting with the anti-GPIIb-IIIa monoclonal antibody (LJ-P4), which is directed against platelets.¹⁴ Monocyte preparations were free of contamination by eosinophils. Viability was greater than 99% as determined by trypan blue exclusion. The average yield of purified and viable monocytes was 0.5 to 1 × 10⁶ cells per 15 ml of ethylenediaminetetraacetic acid blood.

Stimulation of monocytes in suspension

Monocytes were stimulated with calcium ionophore under conditions previously shown to be optimal.¹¹ Aspirin desensitization did not alter the optimal conditions for stimulation of monocyte eicosanoid release. Aliquots of 5 × 10⁶ monocytes in 1 ml of Hank's balanced salt solution containing 10 μmol/L Ca⁺⁺ were placed in polypropylene tubes and cultured in a water bath at 37° C. They were stimulated by 10 μmol/L Ca⁺⁺ ionophore A23187 (Sigma Chemical Co., St. Louis, Mo), which was dissolved in dimethylsulfoxide and diluted to 0.1% final volume (vol/vol) in culture medium. After 30 minutes of culture, the cells were pelleted by centrifugation (500 *g* for 5 minutes at 4° C), and the supernatants were harvested. The supernatants were immediately frozen in liquid nitrogen and stored

in 1.5 ml Eppendorf tubes (Lincoln Scientific, Santee, Calif.) at -80° C until enzyme immunoassay (EIA) was performed.

Quantification of arachidonic acid metabolites

LTB₄, LTC₄, and TxB₂ (the stable product of TxA₂) were measured in the culture supernatants by direct EIA, as previously described.¹¹ Tracers linked to acetylcholinesterase, antisera, mouse monoclonal anti-rabbit IgG, lyophilized synthetic standards, and the enzyme substrate (Ellman's reagent) were purchased from Cayman Chem. Corp. (Ann Arbor, Mich.). Certified 96-well microtiter plates were purchased from Nunc (Kamstrup, Denmark). The sensitivity of the assays (at 80% bound/maximum bound) varied as follows: LTB₄, 4.5 to 10 pg/ml; LTC₄, 15 to 38 pg/ml; and TxB₂, 7.8 to 18 pg/ml. Cross-reactivity of the antisera as reported by the manufacturer are: antisera to LTC₄ (100%) cross-react with LTD₄ (46%), LTE₄ (2%), and LTB₄ (0.01%); antisera to LTB₄ (100%) cross-react with 5(S),12(S) mono- and di-hydroxy-eicosatetraenoic acid (0.3%), LTC₄ and LTD₄ (0.01%); and antisera to TxB₂ (100%) cross-react with 2,3 dinor TxB₂ (8.2%), prostaglandins (<0.44%), and LTB₄ (0.01%). Specificity of the LTB₄ EIA has been previously confirmed by reverse-phase high-performance liquid chromatography.¹¹

Statistical analysis

All data are expressed as the means ± SEM for triplicate cultures of 5 × 10⁴ cells, each assayed in duplicate. The Mann-Whitney U and Wilcoxon signed-rank nonparametric tests were used for statistical comparisons; *p* values of less than 0.05 were considered significant. All analyses were performed on a Macintosh computer (Apple Computer, Inc., Cupertino, Calif.) with StatView II software (Abacus Concepts, Berkeley, Calif.).

RESULTS

Clinical characteristics

Ten patients with ASRD were studied before, during, and after oral aspirin desensitization. The clinical profile of these patients is shown in Table I. Before the aspirin challenge, eight of the 10 patients with ASRD were taking prednisone (mean dose, 10.4 ± 2.2 mg/day). After desensitization, all patients were receiving maintenance doses of 650 mg of aspirin twice a day. After aspirin had been taken for 2 weeks to 10.5 months (mean interval, 5.1 ± 1.3 months), the average prednisone dose fell significantly to 1.6 ± 2.8 mg/day (*p* = 0.018). Although FEV₁ was not obtained in all patients at the restudy point, it was essentially unchanged from the baseline value despite the decrease in prednisone dose in the six cases in which it was measured.

TABLE II. Eicosanoid release from ionophore-stimulated monocytes

	Eicosanoid release (pg/ml $\pm$ SEM)			p Value*		
	BL	Rx	LD	BL vs Rx	Rx vs LD	BL vs LD
ASRD						
TxB ₂	1060 $\pm$ 245[†] (n = 9)	219 $\pm$ 108 (n = 6)	44 $\pm$ 10 (n = 7)	0.0431	0.0796	0.0117
LTB ₄	861 $\pm$ 139[†] (n = 10)	1194 $\pm$ 251[†] (n = 6)	484 $\pm$ 85 (n = 7)	0.0277	0.0464	0.0218
LTC ₄	147 $\pm$ 31[†] (n = 8)	156 $\pm$ 34 (n = 6)	62 $\pm$ 27 (n = 5)	0.7532	0.2733	0.1380
Normal	(n = 5)	(n = 5)	(n = 5)			
TxB ₂	456 $\pm$ 62	344 $\pm$ 60	25 $\pm$ 8	0.2249	0.0431	0.0431
LTB ₄	341 $\pm$ 44	481 $\pm$ 47	466 $\pm$ 55	0.0431	0.6858	0.1380
LTC ₄	56 $\pm$ 6	108 $\pm$ 17	102 $\pm$ 22	0.0431	0.8927	0.2249

Timing of samples is detailed in the text.

BL, Baseline; Rx, reaction; LD, long-term desensitization.

*Paired two-group analysis by Wilcoxon signed-rank test. Significant values ($p < 0.05$) are in boldface type.

[†]Significant difference between ASRD monocytes and normal monocytes by the Mann-Whitney rank test (unpaired two-group analysis). Significant values ($p < 0.05$) are in boldface type.

Eicosanoid release at baseline and during aspirin reactions

TxB₂, LTB₄, and LTC₄ release were measured from calcium ionophore-stimulated monocytes. All 10 patients with ASRD and five normal volunteers were studied at baseline. Six of the patients with ASRD were also studied at the time of the initial aspirin-provoked bronchospasm. The five normal volunteers were studied after ingestion of 60 mg of aspirin.

At baseline (before aspirin challenge), monocytes from patients with ASRD released significantly increased amounts of TxB₂, LTB₄, and LTC₄ compared with normal monocytes (Table II). At the time of aspirin-induced bronchospasm in patients with ASRD (after ingestion of 6 to 100 mg of aspirin), there was a significant increase in LTB₄ release and a significant decrease in TxB₂ release (Table II).

After ingestion of 60 mg of aspirin in normal volunteers, LTB₄ and LTC₄ release were significantly increased. The acute increase in LT release may result from shunting of arachidonic acid metabolism from the cyclooxygenase pathway into the lipoxygenase pathway. Regression analysis did not show any relationship between baseline values for TxB₂, LTB₄, or LTC₄ release and the initial prednisone dose or the fall in FEV₁ during the aspirin reaction.

Eicosanoid release after aspirin desensitization

Patients with ASRD were considered to be desensitized when they could ingest 650 mg of aspirin without experiencing a significant fall in FEV₁ (greater than 20% from baseline). Monocytes were ob-

tained 3 hours after ingestion of the first tolerated dose of 650 mg of aspirin in seven of the patients with ASRD (acute desensitization time point). Seven of the 10 patients with ASRD also had monocytes obtained after taking 650 mg of aspirin twice a day for a period of 2 weeks to 10.5 months (long-term desensitization time point). These samples were obtained 3 hours after a 650 mg dose of aspirin was taken. No patient experienced significant side effects from the long-term aspirin treatment. Two desensitization samples were also obtained in the normal volunteers: the acute desensitization sample was obtained 3 hours after 650 mg of aspirin was taken for the first time, and the long-term desensitization sample was obtained 3 hours after 650 mg of aspirin was taken twice a day for 8 days.

The amounts of eicosanoids released by monocytes from patients with ASRD and normal control subjects were determined at both desensitization time points. The patterns of TxB₂, LTB₄, and LTC₄ release from monocytes of patients with ASRD and normal monocytes at both the acute and long-term desensitization time points are shown in Figs. 1 to 3, and the statistical comparisons are shown in Table II. As expected, desensitization TxB₂ release was significantly suppressed in both normal monocytes and those of patients with ASRD.

LTB₄ release by ASRD monocytes at the long-term desensitization time point was significantly less than the corresponding values at baseline (Table II). Fig. 2 shows that mean ASRD monocyte LTB₄ release continued to fall between the acute and chronic desensitization time points. By the long-term desensitization time point, there was no difference in LTB₄ release between ASRD and normal monocytes.

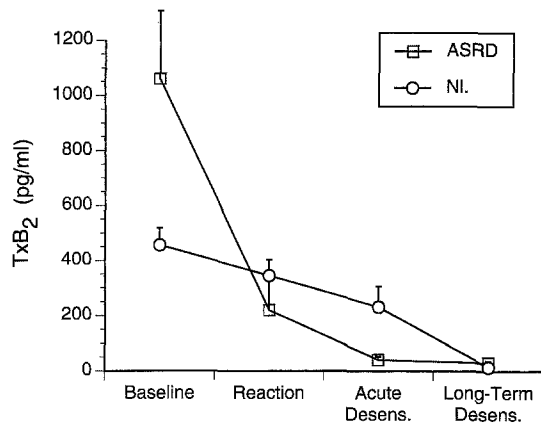


FIG. 1. Impact of aspirin desensitization on the release of TxB₂. Monocytes (5×10^4 /ml) were obtained at the times indicated and stimulated with A23187; TxB₂ release was measured by EIA. Monocytes from patients with ASRD are indicated by *open squares*, and normal monocytes are indicated by *open circles*.

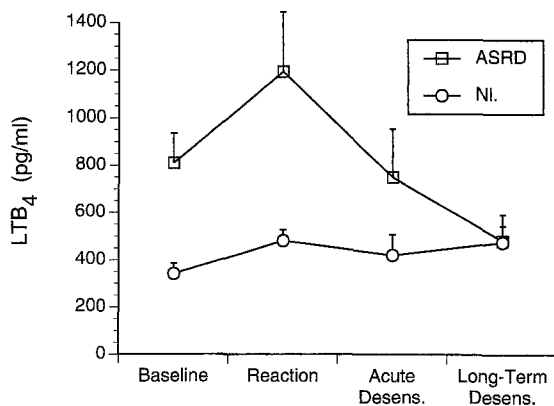


FIG. 2. Impact of aspirin desensitization on the release of LTB₄. Monocytes (5×10^4 /ml) were obtained at the times indicated and stimulated with A23187; LTB₄ release was measured by EIA. Monocytes from patients with ASRD are indicated by *open squares*, and normal monocytes are indicated by *open circles*.

The effects of aspirin desensitization on LTC₄ release were less clear in the five patients in whom LTC₄ release was measured after long-term desensitization. ASRD monocyte LTC₄ release decreased after aspirin desensitization from 196 ± 57 pg/ml in the acute desensitization sample to 62 ± 27 pg/ml in the long-term desensitization sample (Fig. 3). The difference, however, did not reach statistical significance ($p = 0.138$). Four of the patients with ASRD actually released increased LTC₄ at the acute desensitization time point compared with the reaction or baseline time points.

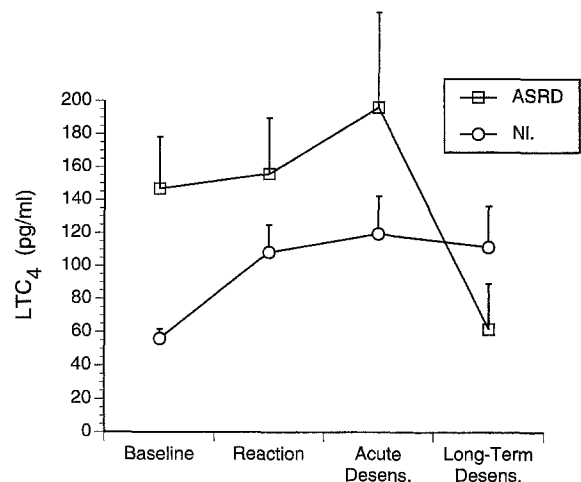


FIG. 3. Impact of aspirin desensitization on the release of LTC₄. Monocytes (5×10^4 /ml) were obtained at the times indicated and stimulated with A23187; LTC₄ release was measured by EIA. Monocytes from patients with ASRD are indicated by *open squares*, and normal monocytes are indicated by *open circles*.

Long-term desensitization samples were obtained from three of these four patients, and in each case the LTC₄ release was less than that released at baseline or the acute desensitization time points.

Normal monocytes released increased amounts of LTB₄ and LTC₄ at the reaction time point but did not show a significant decrease in LT release at the acute or long-term desensitization time points (Figs. 2 and 3 and Table II). Two of the five normal subjects released slightly less LTB₄ or LTC₄ at the long-term desensitization time point compared with baseline values (data not shown), suggesting that a longer course of aspirin therapy might be able to abrogate the acute increase in LT release even in normal volunteers.

DISCUSSION

There is general agreement that inhibition of the cyclooxygenase enzyme in patients with aspirin-sensitive asthma is the common provoking event in aspirin-induced bronchospasm.^{7, 9, 15} Although the mechanism by which cyclooxygenase inhibition results in bronchospasm has not been clearly defined, there is evidence that altered arachidonic metabolism may be a critical feature of the reaction.^{10, 16-19} We previously showed that calcium ionophore-stimulated peripheral blood monocytes of patients with aspirin-sensitive asthma release increased amounts of TxB₂, LTB₄, and LTC₄ compared with those of normal volunteers or asthmatic patients without aspirin sensitivity.¹¹ The ability of patients

with ASRD to be desensitized to aspirin and NSAIDs has been well documented and has been shown to result in clinical improvement.¹⁻⁶ In this report we investigated whether there were alterations in monocyte arachidonic acid metabolism that occurred during acute and long-term aspirin desensitization treatment.

Ten patients with ASRD and five normal volunteer subjects were studied before they received aspirin and at various times after they had begun the aspirin challenge. Monocyte eicosanoid release was determined at baseline, after aspirin-induced bronchospasm (or after ingestion of 60 mg of aspirin in the normal subjects), after the first tolerated dose of 650 mg of aspirin (acute desensitization sample), and after taking 650 mg of aspirin twice a day for at least 8 days (and as long as 10.5 months; long-term desensitization sample). Aspirin desensitization was associated with clinical improvement in the group of patients with ASRD, as shown by a decrease in the mean prednisone dose of 10.4 mg/day to 1.6 mg/day (Table I).

The baseline and aspirin reaction data from patients 1 to 8 have been previously reported.¹¹ ASRD monocytes released significantly more TxB_2 , LTB_4 , and LTC_4 than did normal monocytes before aspirin administration. At the time of aspirin-induced bronchospasm, ASRD monocytes released significantly decreased amounts of TxB_2 and significantly increased amounts of LTB_4 . Monocytes from normal volunteers released significantly increased LTB_4 and LTC_4 after ingestion of 60 mg of aspirin.

Desensitization samples were obtained both immediately after the first tolerated 650 mg dose of aspirin (acute desensitization time point), as well as after regular use of aspirin 650 mg twice a day for periods ranging from 8 days to 10.5 months (long-term desensitization time point). Eicosanoid release was variable at the acute desensitization time point and did not predict the magnitude of the fall in LTB_4 at the long-term desensitization time point. Therefore we concentrated on the results obtained at the long-term desensitization time point.

After aspirin desensitization, both groups demonstrated almost complete inhibition of TxB_2 release. Although LTB_4 and LTC_4 release remained elevated compared with baseline in the normal subjects, the patients with ASRD showed significantly decreased LTB_4 release after aspirin desensitization. After aspirin desensitization, the profile of released eicosanoids from ASRD monocytes

was indistinguishable from that of normal monocytes (Table II).

A significant decrease in LTB_4 release was observed in the patients with ASRD after aspirin desensitization despite a concomitant decrease in systemic glucocorticoid therapy in seven of the 10 patients with ASRD. Interestingly, the single patient with ASRD who failed to show a fall in LTB_4 release after aspirin desensitization was patient 2, whose prednisone dose was tapered from 30 mg/day to zero over the course of the study.

The effect of varying prednisone dose on ex vivo monocyte LT release is uncertain. Glucocorticoids are thought to decrease eicosanoid synthesis through inhibition of cytosolic (group II) phospholipase A_2 (PLA_2) activity.²⁰⁻²² Cytosolic PLA_2 activity is increased by some cytokines. Dexamethasone has been shown to inhibit the increase in PLA_2 mRNA levels in forskolin-stimulated rat smooth muscle cells and to decrease posttranscriptional processing of PLA_2 in tumor necrosis factor-stimulated smooth muscle cells.²² Dexamethasone had only a small (<20%) effect on basal PLA_2 activity in the human epithelial carcinoma line HEP-2 but completely abrogated the tumor necrosis factor- α -induced increase in PLA_2 activity.²³

Although glucocorticoids appear to have a greater negative impact on cyclooxygenase metabolism, several in vivo and in vitro studies have shown that glucocorticoids may also inhibit LT synthesis by human macrophages.^{24, 25} A single dose of prednisone was recently shown to decrease LTB_4 production by alveolar macrophages obtained at 4:00 AM in patients with nocturnal asthma but not in normal control subjects or asthmatic patients without nocturnal asthma.²⁶ The effects of in vivo prednisone on eicosanoid metabolism in normal control subjects has been shown to be cell-specific.²⁷ Administration of prednisone (60 mg/day for 7 days) to normal volunteer subjects did not alter urinary excretion of prostaglandins (2,3-dinor TxB_2 , 11-dehydro- TxB_2 , prostaglandin I_2 , and prostaglandin E_2) or LTs (LTE_4). LTB_4 release from stimulated (anti-IgE, N-formyl-methionyl-leucyl-phenylalanine, and opsonized zymosan) peripheral blood leukocytes was also not affected by the prednisone. In contrast, in vivo or in vitro exposure to prednisone markedly decreased baseline and stimulated eicosanoid release from bronchoalveolar macrophages.

Although we cannot predict with certainty whether the tapering of prednisone dose during aspirin desensitization altered eicosanoid release

from the peripheral blood monocytes, it is clear that any potential effect would have been to increase the release of LTs. Therefore the observed fall in LTB₄ release in the face of decreasing prednisone doses could not be a direct effect of the change in prednisone dose.

Aspirin desensitization also decreased LTC₄ release from ASRD monocytes, but this effect was not statistically significant (Table II). The downward trend in LTC₄ release was seen between the acute and long-term desensitization samples (Fig. 3). A larger sample size at the long-term desensitization point might have demonstrated a significant fall in LTC₄ release. Because LTC₄ is not a major eicosanoid product in human monocytes,²⁸ this model may not be optimal to study the effects of aspirin desensitization on LTC₄ synthesis. Measurement of urinary LTE₄ levels in patients with ASRD has revealed increased LTE₄ excretion compared with normal control subjects with a significant increase after aspirin challenge.^{17, 29, 30} The effects of aspirin desensitization on urinary LTE₄ excretion have been reported in a single patient with ASRD who was shown to excrete progressively decreasing amounts of urinary LTE₄ during a 3-week period of maintained aspirin desensitization.³¹

Patients with ASRD were discharged from the hospital and returned to their homes throughout the United States immediately after acute desensitization. It was not possible, therefore, to standardize the timing of the long-term desensitization sample or to obtain long-term desensitization samples in all subjects. The timing of the long-term desensitization sample ranged from 2 weeks to 10.5 months after desensitization in the patients with ASRD but was uniformly obtained at 8 days in the normal subjects. Although this variability could introduce a source of bias in the results, there was no correlation observed between duration of aspirin administration and LT inhibition in the patients with ASRD. In fact, the patient with ASRD (no. 4) with the shortest duration of aspirin administration displayed the greatest percentage decrease in LTB₄ (84%) and LTC₄ (82%) release. It is possible, however, that longer periods of aspirin therapy in normal individuals could result in a significant fall in LT release compared with baseline values.

The mechanism by which aspirin desensitization results in decreased monocyte LTB₄ release is unknown. Potential mechanisms for this inhibition include: decreased 5-lipoxygenase or LTA hydrolyase activity, decreased arachidonic acid availability, increased competition for arachidonic acid, or

increased LT catabolism. Although NSAIDs are primarily inhibitors of cyclooxygenase, they have been shown to inhibit lipoxygenase in some situations. Bromfenac and indomethacin decreased LTB₄ levels in human follicular fluid,³² whereas aspirin and other NSAIDs inhibited LTC₄ formation by ethanol-stimulated rat gastric mucosa.³³ At high doses (>1 mmol/L) aspirin has been shown to affect the 12-lipoxygenase pathway, inhibiting the metabolism of 12-hydroxyperoxy-eicosotetraenoic acid to 12-hydroxy-eicosatetraenoic acid by an action on the 12-hydroxyperoxy-eicosotetraenoic acid peroxidase.³⁴ When added to neutrophils *in vitro*, high doses of indomethacin (>100 μmol/L) significantly inhibited calcium ionophore-stimulated LTB₄ release.³⁵ The possibility exists that prolonged exposure to therapeutic levels of aspirin (<1 mmol/L) can inhibit lipoxygenase enzymes. On the basis of the high specificity of aspirin for cyclooxygenase relative to lipoxygenase, this possibility appears unlikely.

Alternatively, aspirin desensitization might act indirectly to decrease monocyte LT release. Both cyclooxygenase and lipoxygenase activity are influenced by the *in vivo* milieu, including exposure to cytokines such as interleukin-1, interferon-γ, and granulocyte-macrophage colony-stimulating factor.³⁶ In addition to cyclooxygenase inhibition, NSAIDs have been shown to have other effects, including inhibition of neutrophil activation³⁷ and inhibition of the interleukin-1-mediated induction of cyclooxygenase mRNA and protein expression.³⁸ Thus one could speculate that NSAIDs might alter the *in vivo* inflammatory environment in a way that could downregulate LT synthesis. For example, prostaglandin D₂ has been shown to enhance LTC₄ release by human eosinophils.³⁹

Irrespective of the mechanism of action, aspirin desensitization was associated with decreased LTB₄ release and clinical improvement, which included control of asthma and rhinitis while significantly decreasing the requirement for systemic corticosteroids. LTs have potent bronchospastic and inflammatory actions.^{36, 40-42} Patients with ASRD have recently been shown to be hyperresponsive to inhalation of LTs,¹⁶ and the LT inhibitor SK&F 104353 blocked aspirin-induced bronchospasm.¹⁸ These observations suggest that the inhibition of LT release found in aspirin-desensitized patients could be directly related to their reduced need for antiinflammatory corticosteroid therapy.

The effects of regular aspirin treatment on LT release from monocytes of non-aspirin-sensitive

asthmatic patients was not investigated in this study. We have previously demonstrated that LT release from non-aspirin-sensitive asthmatic patients was indistinguishable from that of normal control subjects,¹¹ making it unlikely that an effect of aspirin would be detectable without use of a large patient population.

In summary, this report describes decreased LTB₄ release from calcium ionophore-stimulated monocytes of patients with ASRD after aspirin desensitization. On the basis of the biologic actions of LTs in the airway, as well as the sensitivity of patients with ASRD to LTs, inhibition of LT synthesis could account for the clinical benefit of aspirin desensitization. It will be important to extend these results in an analysis of a larger group of patients with ASRD to determine whether the change in LT release correlates with the degree of clinical improvement.

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