ORIGINAL ARTICLE

Unique Effect of Aspirin Therapy on Biomarkers in Aspirin-exacerbated Respiratory Disease

A Prospective Trial

Katherine N. Cahill[1], Jing Cui[2,3], Parul Kothari[2,3], Katherine Murphy[2], Benjamin A. Raby[3,4,5], Joseph Singer[2], Elliot Israel[2,3,4], Joshua A. Boyce[2,3], and Tanya M. Laidlaw[2,3]

[1]Division of Allergy, Pulmonary and Critical Care Medicine, Department of Medicine, Vanderbilt University, Nashville, Tennessee; [2]Division of Rheumatology, Immunology, and Allergy and [4]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts; [3]Harvard Medical School, Boston, Massachusetts; and [5]Channing Division of Network Biology, Boston, Massachusetts

ORCID ID: 0000-0002-8549-1835 (K.N.C.).

Abstract

Rationale: Daily high-dose aspirin therapy benefits many patients with aspirin-exacerbated respiratory disease but provides no benefit for aspirin-tolerant patients with asthma. Type 2 inflammation characterizes aspirin-exacerbated respiratory disease.

Objectives: To determine whether high-dose aspirin therapy changes biomarkers of type 2 inflammation in aspirin-exacerbated respiratory disease.

Methods: Forty-two subjects with aspirin-exacerbated respiratory disease underwent an aspirin desensitization and were placed on high-dose aspirin (1,300 mg daily). Fifteen aspirin-tolerant subjects with asthma were also placed on high-dose aspirin. Biologic specimens and clinical parameters were collected at baseline and after 8 weeks on aspirin. Urinary eicosanoids, plasma tryptase and cytokine levels, platelet–leukocyte aggregates, and granulocyte transcripts were assessed.

Measurements and Main Results: Eight weeks of high-dose aspirin decreased nasal symptoms and urinary prostaglandin E metabolite ($P < 0.05$) and increased urinary leukotriene E_4 ($P < 0.01$) levels in subjects with aspirin-exacerbated respiratory disease, but not in those with aspirin-tolerant asthma. Urinary prostaglandin D_2 and thromboxane metabolites decreased in both groups. Only in subjects with aspirin-exacerbated respiratory disease, exhaled nitric oxide ($P < 0.05$), plasma tryptase ($P < 0.01$), and blood eosinophil ($P < 0.01$) and basophil ($P < 0.01$) counts increased and plasma tryptase correlated with eosinophil counts (Pearson $r = 0.514$; $P < 0.01$) on aspirin. After correction for eosinophil counts, aspirin-induced changes in blood granulocyte transcripts did not differ between groups. Aspirin had no effect on platelet–leukocyte aggregates, platelet activation markers, or plasma cytokines in either group.

Conclusions: High-dose aspirin therapy for 8 weeks paradoxically increases markers of type 2 inflammation in subjects with aspirin-exacerbated respiratory disease, despite reducing nasal symptoms. This effect of aspirin is unique to aspirin-exacerbated respiratory disease and not observed in subjects with aspirin-tolerant asthma.

Keywords: aspirin-exacerbated respiratory disease; type 2 inflammation; aspirin-tolerant asthma; mast cell; cysteinyl leukotrienes

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Correspondence and requests for reprints should be addressed to Tanya M. Laidlaw, M.D., Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital, Building for Transformative Medicine, 60 Fenwood Road, Suite 5002B, Boston, MA 02115. E-mail: tlaidlaw@bwh.harvard.edu.

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At a Glance Commentary

Scientific Knowledge on the Subject: High-dose aspirin therapy is the only targeted therapy available for aspirin-exacerbated respiratory disease, a phenotype of asthma characterized by mast cell activation and cysteinyl leukotriene production, yet the mechanism of clinical benefit is poorly understood.

What This Study Adds to the Field: In 42 subjects with aspirin-exacerbated respiratory disease, 8 weeks of aspirin 1,300 mg daily paradoxically increased markers of type 2 inflammation, mast cell activation, cysteinyl leukotriene production, peripheral blood eosinophilia, and exhaled nitric oxide, findings not observed in 15 aspirin-tolerant subjects with asthma. These observations support that the exquisite dependence on prostaglandin E_2 inhibition for mast cell activation and 5-lipooxygenase activity is a central defect in aspirin-exacerbated respiratory disease and reveal that the therapeutic benefit of high-dose aspirin is independent of a global change in the prostaglandin E_2 pathway.

Aspirin-exacerbated respiratory disease (AERD) is characterized by late-onset asthma, chronic rhinosinusitis with nasal polyposis, tissue eosinophilia, and pathognomonic acute respiratory reactions upon exposure to all nonselective cyclooxygenase (COX) inhibitors. Aspirin desensitization followed by daily high-dose aspirin therapy slows nasal polyp regrowth and improves respiratory symptoms after 6–12 months and can be offered as a therapeutic option to most patients with AERD (1, 2). Aspirin therapy provides no benefit for patients with aspirin-tolerant asthma (ATA) (3). Despite a working understanding of the chronic baseline and acute COX-1 inihibitor–induced reactions in AERD, the mechanism by which daily aspirin provides therapeutic benefit in AERD and the early biomarkers of clinical response are poorly understood.

At baseline, AERD is characterized by dysregulated eicosanoid pathways leading to profound type 2 inflammation. Impairments in respiratory tract prostaglandin (PG) E_2 production and/or signaling lead to mast cell activation and 5-lipoxygenase activation. Elevated baseline urinary cysteinyl leukotriene (cysLT) and PGD_2 metabolite (PGD-M) levels may reflect markers of an impaired PGE_2 pathway. Platelet–leukocyte aggregates, which are increased at baseline in subjects with AERD, serve as an additional source of cysLTs and may facilitate recruitment of granulocytes to the tissue (4, 5). On COX-1 inhibition, metabolites of the cysLT and PGD_2 pathways, leukotriene E_4 (LTE_4) and PGD-M, respectively, surge above their elevated baselines and correspond with profound upper and lower respiratory symptoms. Reactions also involve mast cell activation (indicated by increases in nasal fluid and/or serum/plasma tryptase [6, 7]) and effector cell (eosinophil and innate lymphoid type 2 cell) recruitment from the peripheral blood into the respiratory tract (8, 9). The impact of high-dose aspirin therapy on these known biomarkers of AERD has not been systematically studied and compared with the response to high-dose aspirin in aspirin-tolerant control subjects.

Previous studies of high-dose aspirin therapy in patients with AERD propose type 1 cysLT receptor downregulation (10), PGD_2 inhibition (8), and IL-4/STAT-6 inhibition (11, 12) as potential mechanisms of the clinical benefit. Despite clear clinical benefits, small studies of subjects with AERD demonstrate that high-dose aspirin may paradoxically increase levels of serum tryptase and urinary LTE_4 (8, 11). In a clinical trial of high-dose aspirin (650 mg twice daily) in subjects with ATA and AERD we tested the hypothesis that the clinical benefit of daily high-dose aspirin in AERD is independent of a reduction in mast cell activation and cysLT production. The results from this phase of the clinical trial have not been previously reported. The primary endpoints from the prasugrel crossover phase of the clinical trial (Figure 1A) (NCT01597375) were negative and were previously reported (13).

Methods

After a 4-week run-in period on stable asthma treatment and montelukast (10 mg daily), subjects with ATA and AERD were placed on 650 mg of aspirin twice daily for 8 weeks. Subjects with AERD underwent a 1-day aspirin desensitization protocol as previously reported (13) and ATA subjects underwent a single-dose 325-mg aspirin challenge. Each subject's baseline asthma treatment regimen including montelukast at stable dosages was continued throughout the duration of the study period. Before exposure to aspirin, baseline blood, urine, and lung function were collected in all subjects, and fractional exhaled nitric oxide (Fe_{NO}) was collected in subjects with AERD (Figure 1A). Symptomatic response to high-dose aspirin therapy was assessed by the seven-item asthma control questionnaire (ACQ-7) with lower values denoting better control and a minimally important difference of 0.5 (14, 15) and the total nasal sinus symptom (TNSS) score, a composite score of nasal congestion, runny nose, itchy nose, sneezing, itchy eyes, teary eyes, itchy ears or throat, or eye redness (0–5 for each question, maximum 40 with a lower value denoting less severe symptoms) (13).

Participants with AERD had a history of physician-diagnosed asthma and nasal polyposis, and clinical reactions to oral aspirin with features of upper and/or lower airway involvement confirmed by formal aspirin challenge (13). They met clinical qualifications for high-dose aspirin therapy with respiratory symptoms that were not adequately responsive to other standard therapies, including inhaled and intranasal corticosteroids and leukotriene modifiers. Participants with ATA had physician-diagnosed asthma, no current nasal polyposis, and no history of adverse reaction to any nonselective COX inhibitor. Both groups had stable asthma, defined as a post-bronchodilator FEV_1 of greater than or equal to 70% of predicted, no increase in baseline dose of oral glucocorticoids for at least 3 months, and no history of hospitalization or emergency room visits for asthma for at least the prior 6 months. Patients were excluded if they had current severe gastroesophageal reflux disease, a history of peptic ulcer disease, gastrointestinal bleed or bleeding diathesis, or current use of anticoagulant or any antiplatelet drugs. All patients were between the ages of 18 and 65 years old, were nonpregnant, non-breast-feeding, were not current smokers, and signed informed consent. This was a single-site study, conducted at the Asthma Research

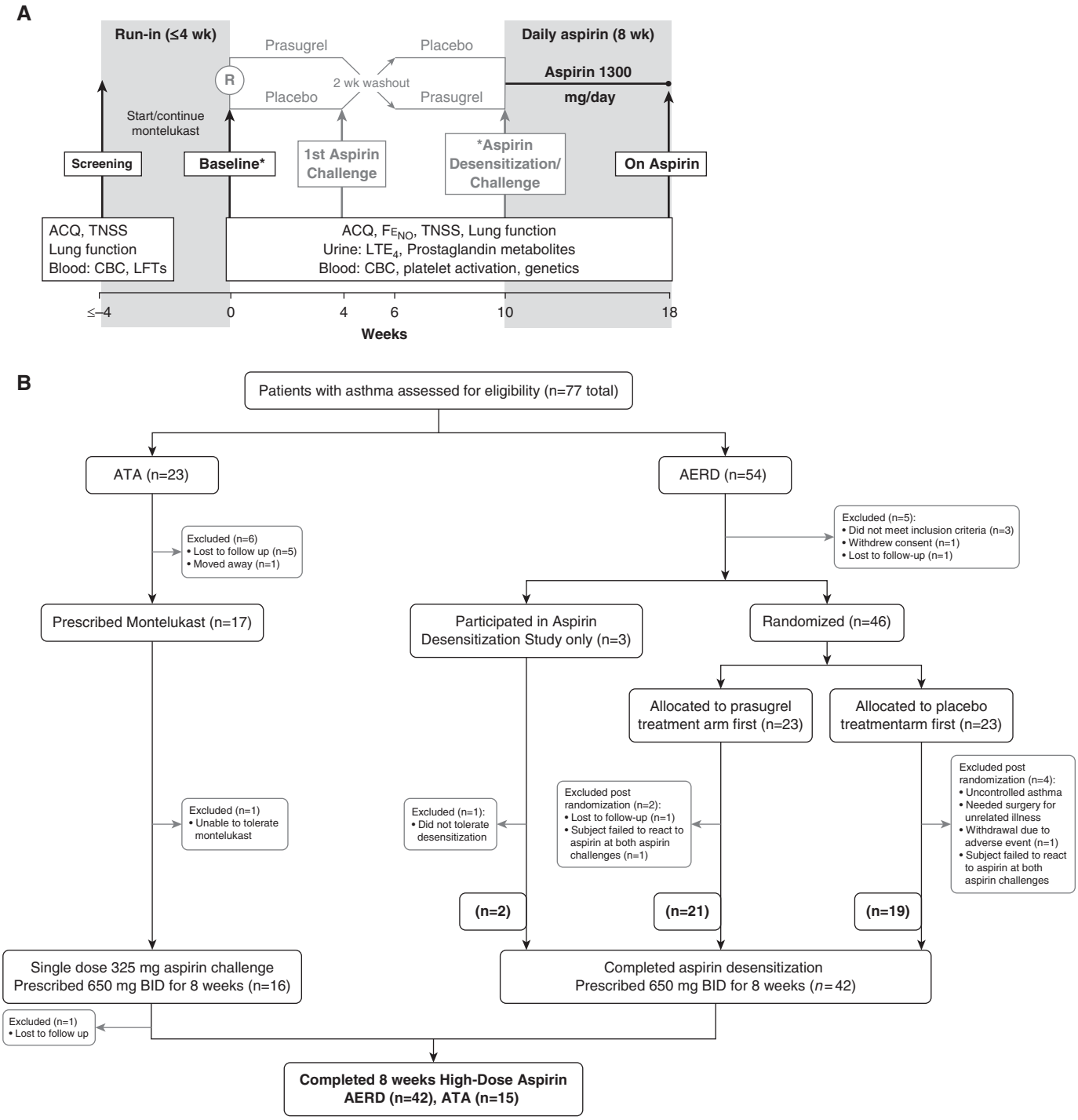

Figure 1. Trial design. (*A*) Overview of the trial design and procedures. The period between screening and V1 was a minimum of 24 hours (for patients already taking montelukast) and a maximum of 4 weeks. At the baseline visit, baseline disease symptoms including asthma control questionnaire and total nasal symptom scores, lung function, and fractional exhaled nitric oxide were assessed, and blood and urine samples were collected. Most patients with aspirin-exacerbated respiratory disease (AERD) then underwent randomization to prasugrel or placebo and underwent two aspirin challenge/desensitization visits (gray font) and completed desensitization at Week 10. *The remainder completed aspirin challenge (all patients with aspirin-tolerant asthma [ATA]) and desensitization (remaining patients with AERD) at the baseline visit at Week 0. All subjects who successfully completed challenge and/or desensitization returned on high-dose aspirin 8 weeks later. (*B*) Summary of the numbers of patients involved in screening, randomization, and study completion for subjects with AERD and ATA. ACQ = asthma control questionnaire; BID = twice a day; CBC = complete blood count; FE_{NO} = fractional exhaled nitric oxide; LFT = liver function test; LTE_4 = leukotriene E_4; TNSS = total nasal symptom scores.

Center of the Brigham and Women's Hospital and approved by the local institutional review board.

Urinary Measurements

All urine samples were stored at −80°C and were analyzed by gas chromatography–mass spectrometry at Vanderbilt University as previously described (4). Concentrations of LTE_4, the major urinary thromboxane metabolite 11-dehydrothromboxane B_2 (TXB-M) (16), the major PGD_2 metabolite 9a,11b-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (PGD-M) (17), and the PGE metabolite 9,15-dioxo-11a-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (PGE-M) were measured and reported as picomoles per milligram of creatinine.

Flow Cytometry

Peripheral blood was drawn into heparinized tubes, kept at room temperature, and processed or assayed within 1 hour of collection. Complete blood counts with differential were processed at a reference laboratory (LabCorp). Platelet-rich plasma was obtained from the top layer after a 20-minute centrifuge at 200 × *g*. Platelet-rich plasma was incubated with directly conjugated antibodies specific for CD61 and CD62P and whole blood was incubated with directly conjugated antibodies specific for CD61, CD45, CD14, and CCR3, or appropriate isotype controls (BD Biosciences). Cells were fixed in 1% paraformaldehyde and at least 20,000 $CD45^+$ cells for whole blood analyses or 50,000 platelets for platelet-rich plasma analyses were recorded for each sample an a FACSAria flow cytometer (BD Biosciences) and analyzed using FlowJo Version 10 (TreeStar).

Plasma Protein Measurements

Plasma processed within 1 hour of collection was assayed for total and mature tryptase analysis performed at Virginia Commonwealth University by UniCAP (18, 19). Plasma samples were assayed for granulocyte-macrophage colony–stimulating factor, IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12p70, IL-13, IL-17A, monocyte chemoattractant protein 1, macrophage inflammatory protein (MIP) 1α, MIP1β, and tumor necrosis factor-α using a multiplex immunoassay (Fireplex, Abcam) and total IgE by an ELISA (Invitrogen).

Peripheral Blood Granulocyte RNA Sequencing

The 75-bp paired-end reads RNA sequencing of patient-derived peripheral blood granulocytes was performed at the Partners Personalized Medicine Translational Genomics Core. Libraries were prepared using the Illumina TruSeq RNA library preparation kit (Illumina, Inc.) and run on the Illumina HiSeq system. Following adapter trimming with Skewer, reads were aligned to the GRCH38 human genome build with STAR. Quality control for the sequencing reads was done using FastQC. Samples were excluded if they had less than 10 million total reads or there was a discrepancy between *XIST* and Y chromosome expression and reported sex. Differential expression analysis of autosomal transcripts was performed using the "DESeq2" Bioconductor package. Statistical significance was declared at a Benjamini-Hochberg adjusted *P* value less than 0.05.

Outcome Measures

Primary outcomes included change in urinary LTE_4, PGD-M, PGE-M, TXB_2, Fe_{NO}, plasma tryptase, peripheral blood granulocyte counts, platelet–leukocyte aggregates, and COX-2 expression in peripheral blood granulocytes from baseline after 8 weeks of 1,300 mg of aspirin daily, compared between AERD and ATA groups. Clinical outcomes included change in FEV_1% of predicted, TNSS, and ACQ-7 in both groups. Exploratory outcomes included association among urinary eicosanoid levels, mast cell activation as measured by plasma tryptase, and markers of eosinophilic inflammation including peripheral blood granulocyte counts and Fe_{NO}.

Statistical Analyses

Data are expressed as means ± SD unless otherwise noted. Two-sided paired Student's *t* test assessed change on high-dose aspirin from baseline for both groups. A Student's *t* test assessed for a difference in the change on aspirin between the AERD and ATA groups. Correlation between biomarkers on high-dose aspirin was assessed using Pearson correlation coefficient. Analysis was performed using GraphPad Prism version 7.03 for Windows (GraphPad Software) and SAS 9.4.

Results

Clinical Outcomes

Forty-nine subjects with suspected AERD underwent aspirin challenge and attempted aspirin desensitization. Forty-seven subjects demonstrated a positive clinical reaction to aspirin and 42 went on to tolerate high-dose aspirin therapy for 8 weeks (Figures 1A and 1B). Twenty-three ATA subjects were screened and 15 completed an observed single-dose aspirin challenge and returned on high-dose aspirin therapy after 8 weeks (Figure 1B). Subjects with AERD were older, had higher baseline peripheral blood eosinophil counts, and better asthma control as measured by ACQ-7 compared with ATAs (Table 1). Each group used similar amounts of inhaled corticosteroids, whereas subjects with AERD were more likely to be treated with a long-acting β-agonist. There was no difference in sex, race, ethnicity, body mass index, baseline lung function, or TNSS between groups.

On high-dose aspirin, TNSS decreased in subjects with AERD (4.3 ± 0.7 to 2.2 ± 0.6; $P < 0.05$), whereas no change was noted in ATAs, despite similar baseline TNSS scores in the ATA subjects. There was no change in FEV_1% predicted or ACQ-7 in either group. Fe_{NO} increased significantly on high-dose aspirin (from 46.2 ± 26.8 to 64.2 ± 42.6; $P < 0.0$) in subjects with AERD (Table 2). Fe_{NO} was not assessed in ATA.

Urinary Eicosanoids

Eicosanoid levels were assessed in the urine at baseline and after 8 weeks of high-dose aspirin in both subjects with AERD and ATA. High-dose aspirin decreased urinary (u) PGE-M and increased $uLTE_4$, the stable end metabolite of the cysLTs, in subjects with AERD but not ATA, while suppressing uPGD-M and $uTXB_2$ similarly in both groups (Figure 2). Change in uPGD-M, uPGE-M, and $uLTE_4$ from baseline did not correlate with change in TNSS, ACQ-7, FEV_1% predicted, or Fe_{NO} (*see* Table E1 in the online supplement).

Peripheral Blood Cells

At baseline, the subjects with AERD demonstrated a trend toward higher absolute peripheral blood eosinophil counts as compared with ATA (Figure 3), which further increased on high-dose aspirin as previously reported (8). In this larger

Table 1. Patient Demographics

	All AERD ($n = 47$)	ATA ($n = 16$)	*P* Value
Age, yr	47.0 ± 9.2	34.4 ± 15.3	<0.0001
Sex, F	27 (57)	12 (75)	NS
Race			
White	43 (92)	12 (75)	NS
Ethnicity			
Hispanic	3 (6)	2 (13)	NS
Body mass index	28.6 ± 6.0	26.8 ± 4.7	NS
FEV_1, L	3.06 (0.82)	2.95 (0.56)	NS
FEV_1% predicted	91.2 ± 12.5	86.7 ± 10.9	NS
Fraction of exhaled nitric oxide, ppb	46.1 ± 26.8	DNC	
Peripheral eosinophil count, /μl	0.40 ± 0.33	0.23 ± 0.11	<0.05
Total IgE, ng/ml	795 ± 1,370	1,062 ± 1,370	NS
ACQ-7	0.68 ± 0.59	1.21 ± 0.36	<0.01
TNSS	4.3 ± 4.6	2.2 ± 2.9	0.12
Low ICS dose (≤200 μg*)	17 (36)	5 (33)	
Medium ICS dose (201–500 μg*)	19 (40)	5 (33)	
High ICS dose (>500 μg*)	4 (9)	1 (7)	
Oral glucocorticoid use	2 (4)	0	
Long-acting β-agonist use	30 (64)	4 (27)	<0.05

Definition of abbreviations: ACQ = asthma control questionnaire; AERD = aspirin-exacerbated respiratory disease; ATA = aspirin-tolerant asthma; DNC = data not collected; ICS = inhaled corticosteroid; NS = not significant; TNSS = total nasal symptoms score.
Values are *n* (%) or means ± SD.
*Fluticasone proprionate dry powder equivalent.

cohort of subjects, basophils also increased, whereas neutrophils, monocytes, and platelets remained unchanged (Figure 3). Aspirin therapy had no effect on peripheral blood leukocyte counts in ATA. Although we confirmed subjects with AERD had greater numbers of platelet–leukocyte aggregates as compared with ATA at baseline (Figure 4) (4, 5), there was no change in numbers of platelet–leukocyte aggregates on high-dose aspirin in either group (Figure 4), or in platelet activation as assessed by CD62P (data not shown). Eosinophil counts on high-dose aspirin in subjects with AERD correlated weakly with FE_{NO} levels (Pearson correlation coefficient $r_p = 0.314$; $P = 0.07$; data not shown).

Mast Cells

No difference in baseline plasma tryptase was observed between subjects with ATA and AERD. Plasma total tryptase increased and correlated with peripheral blood eosinophil counts (Pearson $r = 0.514$; $P < 0.01$) (Figures 5A and 5B) but not with $uLTE_4$, uPGD-M, or uPGE-M (*see* Figures E1A and E1B) in subjects with AERD on high-dose aspirin. Mature tryptase, reflecting processing associated with mast cell degranulation (18), was not detected at baseline or on high-dose aspirin in either group (data not shown). Aspirin had no effect on total tryptase levels in ATA subjects (Figure 5A).

Table 2. Change in Clinical Parameters on High-Dose Aspirin

	AERD	ATA
FEV_1, %	−0.76 ± 1.78	1.41 ± 1.73
FE_{NO}, ppb	15.8 ± 5.8*	DNC
ACQ-7	−0.11 ± 0.11	−0.17 ± 0.19
TNSS	−1.63 ± 0.73†	−0.79 ± 0.97

Definition of abbreviations: ACQ = asthma control questionnaire; AERD = aspirin-exacerbated respiratory disease; ATA = aspirin-tolerant asthma; DNC = data not collected; FE_{NO} = fractional exhaled nitric oxide; TNSS = total nasal symptoms score.
Data are expressed as means ± SEM.
*$P < 0.01$.
†$P < 0.05$.

Plasma Cytokines

No change in cytokine levels was observed in either AERD or ATA subjects on daily aspirin. Of the assays performed, levels of IL-5, IL-6, IL-8, IL-9, monocyte chemoattractant protein 1, MIP1α, MIP1β, and tumor necrosis factor-α were consistently detected in most samples, with no trends toward differences across time points. Levels of granulocyte-macrophage colony–stimulating factor, IL-10, IL-1β, IL-12p10, IL-13, IL-7A, and INF-γ were detected in none or only a few of the samples. Total IgE levels remained unchanged on high-dose aspirin in both groups (data not shown).

Peripheral Blood Granulocyte RNA Sequencing

Because aspirin is a known acetylator that could influence gene expression (20), we analyzed RNA-sequencing data from peripheral blood granulocytes between subjects with AERD ($n = 25$) and ATA ($n = 10$) to look for changes in gene expression attributable to disease, adjusting for age, sex, and race (*see* Table E2). Forty-five transcripts were differentially expressed at an adjusted *P* less than 0.05. However, only one transcript, *EFHC1*, remained statistically significant (adjusted $P < 0.05$) after correcting for peripheral blood eosinophil counts. No differential expression was observed when the analysis was restricted to the 29 subjects (19 with AERD, 10 with ATA) with normal eosinophil counts (<500 cells/μl). We then evaluated the effect of high-dose aspirin in AERD by analyzing the subset of 15 subjects who had analyzable RNA-sequencing data at baseline and following aspirin therapy. In a model that accounted for the paired data and treatment group, we found 379 transcripts that were differentially expressed following aspirin treatment, including *CYSLTR2*, *RNASE3*, and *ORMDL3* (*see* Table E3). After adjustment for eosinophil counts, there were no differentially expressed genes, suggesting that the bulk of the observed signal likely reflects treatment-induced changes in granulocyte composition.

Discussion

High-dose aspirin therapy is currently the only disease-specific therapeutic that

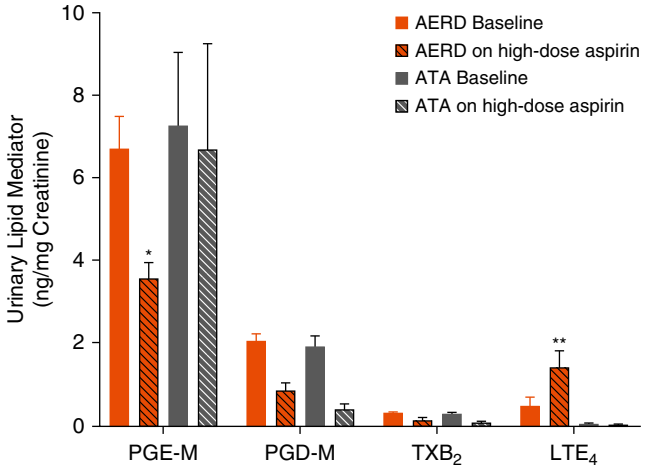

Figure 2. High-dose aspirin suppresses prostaglandin E_2 metabolite and increases cysteinyl leukotrienes in subjects with aspirin-exacerbated respiratory disease (AERD). Urinary eicosanoids were assessed at baseline and after 8 weeks of high-dose aspirin in subjects with AERD and aspirin-tolerant asthma (ATA). *P* values reflect a difference in change from baseline between the AERD and ATA groups. LTE_4 = leukotriene E_4 (AERD $n = 40$, ATA $n = 13$); PGD-M = prostaglandin D_2 metabolite (AERD $n = 43$, ATA $n = 15$); PGE-M = prostaglandin E_2 metabolite (AERD $n = 43$, ATA $n = 15$); TXB_2 = thromboxane B_2 (AERD $n = 41$, ATA $n = 15$). Data are expressed as means + SEM. $^*P < 0.05$ and $^{**}P < 0.01$.

modifies the progression of nasal polyposis, decreases topical and systemic steroid requirements, and improves quality of life for patients with AERD. These benefits are observed in 67–87% of patients with AERD treated with high-dose aspirin for 6–12 months (1), but high-dose aspirin provides no therapeutic benefit to patients with ATA (3). The mechanism of the benefit of daily aspirin therapy in patients with AERD remains poorly understood with no available surrogate biomarker of response. Baseline impairments in PGE_2 production and/or receptor signaling are well described in multiple cell types, across multiple studies in subjects with AERD (21–24).

In 42 carefully phenotyped subjects with AERD, we observed that 650 mg of aspirin twice-daily for 8 weeks failed to correct any of the known baseline derangements in PGE_2 production, cysLT overproduction (Figure 2), and mast cell activation (Figure 4A). In fact, the high-dose aspirin-induced reduction in PGE-M levels (reflecting the anticipated pharmacologic effect of aspirin on COX function) was associated with greater systemic markers of type 2 inflammation: increased total mast cell activation (Figure 4B), a further increase in urinary LTE_4 (Figure 2), the stable end metabolite of the cysLTs, and a doubling of peripheral blood eosinophil counts (Figure 3). The sustained urinary PGE-M levels observed after 8 weeks of high-dose aspirin therapy in subjects with ATA, although surprising, are in agreement with some studies of long-term aspirin use in animals and humans, and potentially reflect increased COX-2 expression induced by aspirin in some cell types (25–28). These observations validate PGE_2 as an important regulator of mast cell activation and 5-lipoxygenase activity, and demonstrate that the failure to induce COX-2, and subsequently sustain PGE_2, is a central defect in AERD. However, they also reveal that the therapeutic benefit of high-dose aspirin is independent of a global change in the PGE_2 pathway.

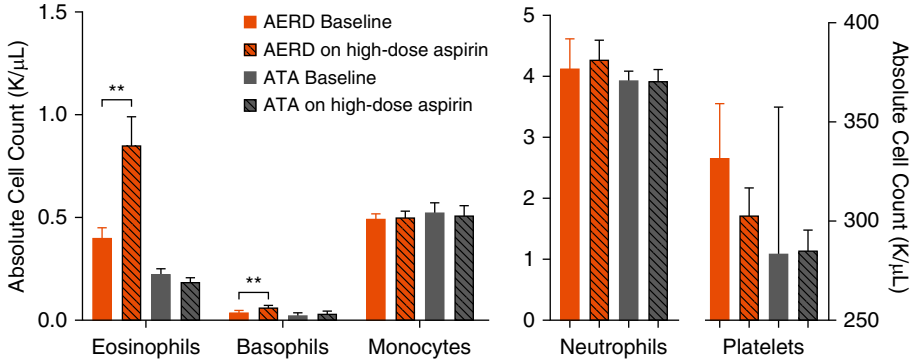

Figure 3. High-dose aspirin increases $CRTH2^+$ peripheral blood cells in aspirin-exacerbated respiratory disease (AERD). Peripheral blood leukocyte counts at baseline and at 8 weeks on high-dose aspirin in subjects with AERD and aspirin-tolerant asthma (ATA). No difference in baseline eosinophil, basophil, monocyte, neutrophil, or platelet counts between ATA and AERD were observed. High-dose aspirin resulted in marked elevation of peripheral blood eosinophil (415–879/μl; $P < 0.01$) and basophil (43–66/μl; $P < 0.01$) counts, whereas no change was noted in neutrophils, monocytes, or platelets. Data are expressed as means + SEM. $^{**}P < 0.01$.

Systemic mast cell activation, as assessed by plasma tryptase, increases in the peripheral blood of subjects with AERD who are on high-dose aspirin (Figure 5A), but not in subjects with ATA. Previous studies have observed an increase in sputum tryptase in subjects with AERD treated with daily aspirin (11). Our data suggest that increases in both mast cell activation and/or burden and cysLT generation occur, whereas total body COX products (PGE-M, PGD-M, and TXB_2) are suppressed on high-dose aspirin. Furthermore, the increase in plasma tryptase levels we demonstrate correlates with a rise in PGD_2-responsive $CRTH2^+$ eosinophils and basophils in the peripheral blood (Figure 5B), despite the suppression in urinary PGD_2 metabolites (Figure 2). We suspect that although the depletion of PGE_2 may allow for increasingly leaky mast cells (reflected in the increases in tryptase), the high-dose aspirin-induced suppression of PGD_2 leads to a loss of the chemotactic gradient that drives eosinophils and basophils into the respiratory tissues. Notably, FE_{NO} (commonly used as a surrogate of eosinophilic inflammation) significantly increased on high-dose aspirin (Table 2). Given that inhalation challenges with PGE_2 and $PGF_2\alpha$ decrease exhaled FE_{NO}

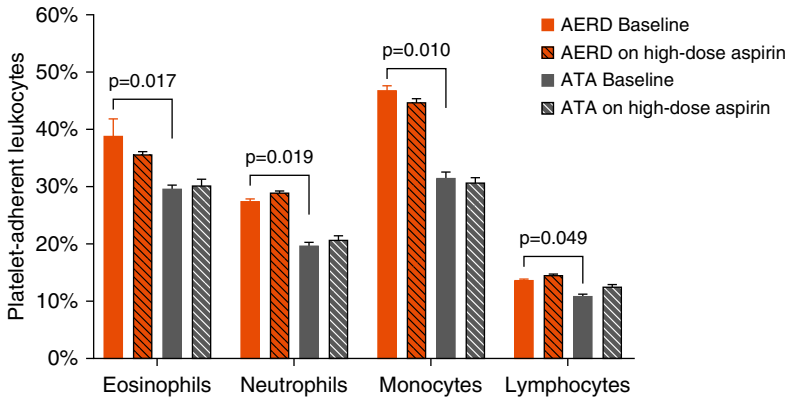

Figure 4. Platelet–leukocyte aggregates are increased in the blood of patients with aspirin-exacerbated respiratory disease (AERD) at baseline and do not change with 8 weeks of high-dose aspirin therapy. Percentages of leukocytes with adherent platelets (as determined by staining with CD61) in blood of aspirin-tolerant asthma control subjects ($n = 15$) and subjects with AERD ($n = 36$). Data are expressed as means + SEM. ATA = aspirin-tolerant asthma.

in healthy humans and subjects with asthma (29), it seems likely that the effect of aspirin on Fe_{NO} reflects a direct effect of endogenous COX products on epithelial cells or other cells that express inducible nitric oxide synthase.

High-dose aspirin therapy increases cysLT production while having no effect on the levels of platelet–leukocyte aggregation or platelet activation seen in the blood of subjects with AERD. Any residual effects of prasugrel exposure 8–14 weeks before the assessments on 8 weeks of daily aspirin in subjects with AERD, although unlikely given the drug half-life of approximately 7 hours, cannot be ruled out. Although mast cells may be a primary source of increased cysLTs in patients with AERD on high-dose aspirin, a global loss of the PGE_2 needed to control 5-lipoxygenase function could also increase the throughput of LTA_4 into other granulocytes and platelets, thereby providing the existing platelet–leukocyte aggregates more substrate to convert to cysLTs. A reduction in end-organ responsiveness (10), or desensitization to excessive cysLT production, has been offered as an explanation for the mechanism of desensitization and tolerance of daily aspirin therapy and has been suggested separately in smaller cohorts of patients. Our current study, with a much larger cohort and direct comparison with ATA subjects, supports and strengthens the previous findings.

Clinical experience has shown that patients who fail to tolerate daily aspirin therapy because of side effects of gastrointestinal symptoms, rash, or worsening lung function typically present with these side effects within the first few weeks of therapy. The increase in mast cell activation and elevation of cysLTs observed in this study may not be tolerated by all patients, which may explain why some patients are unable to continue daily aspirin therapy. The modest clinical benefit, a reduction in nasal symptoms, we observed after 8 weeks of daily aspirin therapy is consistent with our clinical experience and prior publications. The full benefit from daily aspirin therapy can take 6–12 months (1) to be readily clinically evident and is primarily evident as a reduction in nasal polyp regrowth, glucocorticoid use, sinusitis, and/or asthma exacerbations. This study was inadequately powered to report on these late clinical endpoints.

We have found that further and/or persistent reductions in systemic measurements of PGE_2 levels during daily aspirin therapy are associated with increased markers of type 2 inflammation characterized by mast cell activation, cysLT generation, and peripheral blood eosinophilia. These effects of high-dose aspirin are unique to the patient

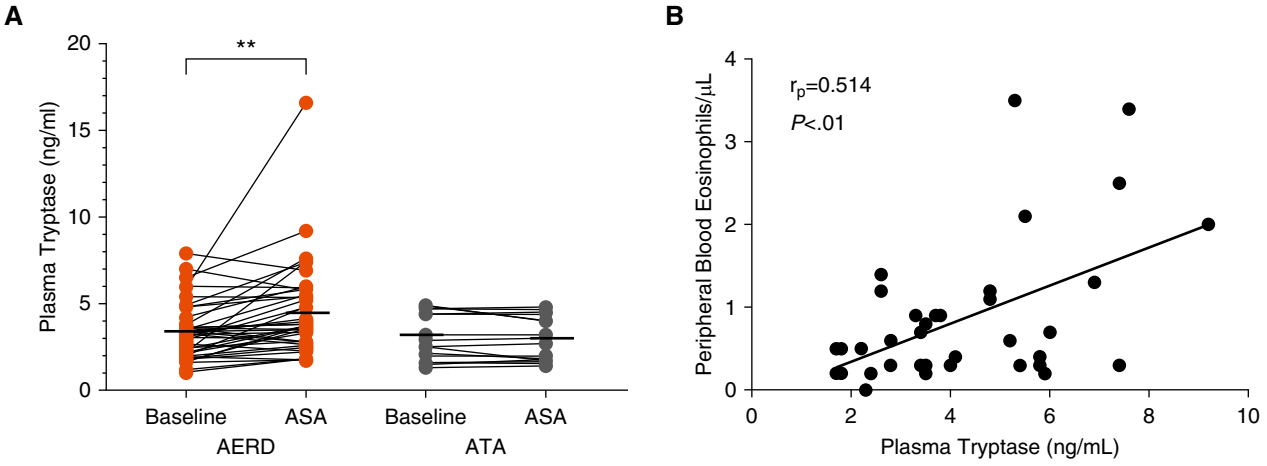

Figure 5. Mast cell activation increases on high-dose aspirin and correlates with peripheral blood eosinophilia observed in aspirin-exacerbated respiratory disease (AERD). (*A*) Plasma total tryptase at baseline and on 8 weeks of high-dose aspirin in subjects with AERD and aspirin-tolerant asthma. Horizontal bars reflect group mean. $^{**}P < 0.01$. (*B*) Plasma tryptase correlation (Pearson) with peripheral blood eosinophil counts on high-dose aspirin in subjects with AERD. ASA = aspirin; ATA = aspirin-tolerant asthma.

population with AERD and are not seen in patients with ATA. We conclude that high-dose aspirin therapy does not correct the impaired eicosanoid and mast cell homeostasis that characterize the chronic baseline and acute COX-1 inhibitor–induced reaction disease states in AERD. Future studies need to look beyond the known biomarkers of AERD and focus on the local respiratory tract microenvironment to solve this enigma. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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