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Prostaglandin D₂: A dominant mediator of aspirin exacerbated respiratory disease

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Abstract

Background—Aspirin desensitization followed by high-dose aspirin therapy is routinely performed for patients with aspirin exacerbated respiratory disease (AERD). Little is known about the contributions of mediators other than cysteinyl leukotrienes to aspirin reactions and to the therapeutic benefit of high-dose aspirin therapy.

Objective—We investigated differences in urinary eicosanoid metabolites and blood eosinophils in subjects with AERD who tolerate and those who fail aspirin desensitization, and also in AERD subjects who were successfully treated with high-dose aspirin therapy.

Methods—29 subjects with AERD were stratified into those who tolerated aspirin desensitization (Group I) and those who did not (Group II). Urine was analyzed for eicosanoid metabolites at baseline, during aspirin reactions, and on high-dose aspirin therapy. Blood was analyzed for cell differentials at baseline and on aspirin therapy.

Results—Basal prostaglandin D₂ metabolite (PGD-M) (13.6 ± 2.7 vs. 7.0 ± 0.8 pmol/mg Cr; $P < .05$), and thromboxane metabolite (TX-M) levels (1.4 ± 0.3 vs. 0.9 ± 0.1 pmol/mg Cr; $P < .01$) were higher in Group II than in Group I. During aspirin reactions, PGD-M remained unchanged while TX-M (0.7 ± 0.1 pmol/mg Cr; $P = .07$) tended to decrease in Group I. In contrast, PGD-M rose dramatically in Group II (61.3 ± 19.9 pmol/mg Cr; $P < .05$), while TX-M did not change. The decrease in FEV₁ inversely correlated with basal urinary levels of both LTE₄ and PGD-M. Blood eosinophil and basophil levels rose while urinary PGD-M (2.2 ± 0.8 pmol/mg Cr, $P < .001$) decreased on two months of high-dose aspirin therapy in Group I.

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Conclusion—Failure to tolerate aspirin desensitization in a subset of subjects with AERD is associated with PGD₂ overproduction. The increase in blood eosinophils and basophils while on high-dose aspirin therapy may reflect the functional consequences of decreased PGD₂ release and the therapeutic benefit of aspirin.

Keywords

Aspirin exacerbated respiratory disease; Samter's triad; Nasal polyps; Asthma; Prostaglandin D₂; Thromboxane; Aspirin desensitization; Cysteinyl leukotrienes; Urinary eicosanoids; Eosinophils

INTRODUCTION

Aspirin exacerbated respiratory disease (AERD) is characterized by asthma, eosinophilic nasal polyposis, and respiratory reactions upon ingestion of cyclooxygenase (COX)-1 inhibitors. The pathogenesis of AERD involves dysregulation of arachidonic acid (AA) metabolism, as indicated by excessive basal generation of the cysteinyl leukotrienes (cysLTs), LTC₄, LTD₄ and LTE₄, which is further increased by COX-1 inhibitors. A long-standing hypothesis for the etiology of the reactions to COX-1 inhibitors in AERD has been that COX-1 inhibition dysregulates 5-lipoxygenase (5-LO), resulting in overproduction of cysLTs. Reactions typically occur at a threshold aspirin dose of 40-160 mg (sufficient to block COX-1 but not COX-2), with symptoms generally confined to the respiratory tract. During reactions, both eosinophils and basophils are rapidly recruited to the respiratory tissue, accompanied by a decline in blood eosinophil levels^{1,2}. The reaction is usually followed by a desensitized state, during which time patients can benefit from high-dose daily aspirin which improves disease control^{3,4}. Neither the mechanism responsible for the recruitment of effector cells nor the basis of the therapeutic benefit of high-dose aspirin are known.

AA is metabolized intracellularly to LTC₄ through the actions of 5-LO and LTC₄ synthase⁵⁻⁷. LTC₄ is released and converted to LTD₄, which in turn is rapidly metabolized to the stable end-metabolite LTE₄ and is excreted in the urine⁸. At least three receptors (CysLT₁, CysLT₂, and GPR99)⁹⁻¹² mediate the actions of cysLTs in vivo. Urinary LTE₄ measurements reflect systemic cysLT production, and basal levels of LTE₄ in the urine of subjects with AERD are higher than those in aspirin-tolerant asthmatic (ATA) controls¹¹. Subjects with AERD who experience large (>30%) decreases in forced expiratory volume in 1 second (FEV₁) during aspirin reactions have both higher basal levels of urinary LTE₄ and greater aspirin-induced increases in LTE₄ than subjects who experience lesser reductions in airflow¹³. Drugs that inhibit CysLT₁ receptors or 5-LO attenuate the severity of respiratory symptoms and blunt the reductions in FEV₁ that occur during aspirin reactions, validating the role of cysLTs¹⁴. Although the efficacy of these drugs justifies their use as prophylaxis for desensitization to aspirin, therapeutic responses to them are not uniform among subjects with AERD, and some experience severe aspirin reactions despite prophylaxis. Therefore it is likely that mediators other than cysLTs contribute to the disease in general and to the reactions to COX-1 inhibitors in particular. However, little is known about these other mediators.

COX-1 and COX-2 convert AA to prostaglandin (PG)H₂, which is converted to PGE₂, PGD₂, PGF₂, thromboxane (TXA₂) and prostacyclin (PGI₂) by cell type-restricted synthases. PGE₂ can induce bronchodilation¹⁵, suppress the activation of mast cells and eosinophils, and block 5-LO activation^{16,17}. While PGE₂ is thought to play a protective role in AERD, less is known about the roles of other COX products. PGD₂, the major COX product of mast cells, and its metabolite 9α,11β-PGF₂, induce bronchoconstriction in asthmatic subjects¹⁸, and asthmatic subjects are hyperresponsive to PGD₂ compared with healthy controls¹⁹. PGD₂ is also a vasodilator²⁰, and is chemotactic for eosinophils, basophils, Th2 cells, and ILC2 cells by acting at chemokine receptor homologous molecule expressed on Th2 lymphocytes (CRTH2), also known as the D prostanoid 2 (DP2) receptor^{21,22}. The plasma levels of 9α,11β-PGF₂^{23,4} in AERD exceed those in ATA and healthy controls, and increase modestly within minutes of aspirin challenge²⁴. Bronchoalveolar lavage (BAL) fluid levels of PGD₂ remain unaffected by endobronchial challenge of subjects with AERD using lysine aspirin, whereas metabolites of other PGs decrease²⁵. These studies suggest that COX function and production of PGD₂ in AERD may be at least partly resistant to aspirin. Although the bronchoconstriction, vasodilation, and inflammation-promoting actions of PGD₂ fit with a role in AERD, its functions in the disease remain largely unexplored.

We identified a subset of patients with AERD who did not tolerate desensitization to oral aspirin. Despite prophylaxis with the CysLT₁ receptor antagonist montelukast, these patients failed to advance beyond a threshold dose of aspirin or had difficulty tolerating escalating doses of aspirin due to ongoing cutaneous and/or gastrointestinal symptoms. We hypothesized that differences in eicosanoid generation may account for the differences in reaction severity and the development of systemic extrapulmonary symptoms in this subgroup. Here we demonstrate that subjects with AERD who are unable to tolerate desensitization display markedly dysregulated PG production, particularly PGD₂, which increases dramatically during their reactions. The production of PGD₂ correlates with the severity of airflow obstruction during clinical reactions. We also show that subjects who are successfully desensitized and then treated with high-dose aspirin exhibit sharply reduced systemic production of PGD₂, but not of cysLTs, and demonstrate that reduced tissue flux of classical effector cells (eosinophils and basophils) is potentially a beneficial consequence of the reduced PGD₂ generation.

METHODS

Patient selection and stratification

Subjects with AERD who underwent aspirin desensitization at Brigham and Women's Hospital (Boston, MA) between 2009 and 2014 and signed informed consent to have urine and/or blood samples collected were included. All subjects had a history of asthma, nasal polyposis and characteristic respiratory reactions upon ingestion of COX-1 inhibitors. All were offered desensitization due to refractory rhinosinusitis and/or nasal polyposis, as specified in the 2007 practice parameter²⁶. Aspirin desensitizations were performed while patients were not receiving the 5-LO inhibitor zileuton so that the production of cysLTs could be monitored. Patients took their regularly prescribed inhaled corticosteroids (ICS)

with or without long-acting beta agonists the morning of desensitization as applicable. ICS use at the time of desensitization was recorded as low, medium, and high dose²⁷. All subjects except two received montelukast (10mg) the evening prior to and the morning of aspirin desensitization to attenuate the severity of respiratory symptoms during the reaction¹⁴. Patients were assigned to either Group I (those who tolerated desensitization, n = 23) or Group II (those who failed to tolerate the procedure due to intractable abdominal pain, rash, or unresolved lower respiratory tract symptoms, n=6). Demographic and clinical data was extracted from the medical record at the time of desensitization.

Control subjects with ATA had a history of physician-diagnosed asthma and had tolerated a COX-1 inhibitor in the past 6 months. All subjects were non-smokers.

Aspirin desensitization protocol

Two subjects from Group I had a history of a previous reaction to non-steroidal anti-inflammatory drug (NSAID) ingestion that required epinephrine administration and underwent aspirin desensitization in our medical ICU; all other subjects underwent desensitization in our outpatient clinic. Oral aspirin desensitizations started with 40 mg of aspirin followed by dose increases (81 mg, 162 mg, 325mg) every 90 minutes²⁸. Patients were observed for respiratory symptoms, ocular injection, flushing, rash and abdominal pain. The aspirin dose that caused upper and/or lower respiratory symptoms was recorded as the provocative dose. FEV₁ for each patient in the outpatient clinic was recorded at baseline, prior to each dose, and at the time of reaction.

Urinary eicosanoid measurements

Basal (pre-aspirin administration) urine samples were collected from all subjects. Urine was also collected for AERD subjects 180 minutes after the onset of aspirin-induced reactions. 14 subjects with AERD also provided urine samples after at least 8 weeks of aspirin 1300 mg daily. ATA subjects provided basal urine samples which were collected off of all NSAIDs for more than 1 week. A subset of ATA subjects (n=5) also had urine collected 3 hours after ingestion of 325 mg of aspirin. All urine samples were stored at -80°C and analyzed via gas chromatography-mass spectrometry (GC-MS) at Vanderbilt University. As described previously, concentrations of LTE₄²⁹, the major urinary thromboxane metabolite 11-dehydrothromboxane B₂ (TXB-M)³⁰, the major prostaglandin D₂ metabolite 9α,11β-dihydroxy-15-oxo-2,3,18,19-tetranorprost-5-ene-1,20-dioic acid (PGD-M)³¹, the prostaglandin E metabolite 9,15-dioxo-11α-hydroxy-13,14-dihydro-2,3,4,5-tetranor-prostan-1,20-dioic acid (tetranor-PGE-M), and the prostacyclin metabolite 2,3-dinor-6-keto-PGF1α (PGI-M), were measured and reported as pmol/mg creatinine.

Peripheral blood leukocyte counts

Basal and on high-dose aspirin blood was collected for complete blood counts (LabCorp, Burlington, NC) in a subset of Group I subjects (n=11).

Statistics

All data presented are the mean + standard error of the mean (SEM) and were normally distributed. Baseline clinical characteristics and signs of the reaction to aspirin were

compared between AERD Group I and Group II subjects using a two-tailed t-test or for categorical data, a Fisher's test. Basal and post-aspirin ingestion eicosanoids from ATA, Group I and Group II subjects were compared using a one-way ANOVA followed by unpaired, two-tailed t-tests if the ANOVA *P*-value was less than .05. *P*-values reported represent t-test results. Basal and reaction eicosanoids for each subject were compared using a paired two-tailed t-test. Pearson's correlation coefficient was used for comparisons between urinary eicosanoid levels and fall in FEV₁ or change in blood eosinophils. For all analyses, a *P* < .05 was considered statistically significant. All analyses were performed using GraphPad Prism version 6.03 for Windows, GraphPad Software, La Jolla, CA, www.graphpad.com.

The Brigham and Women's Hospital human subjects Institutional Review Board approved the study and all subjects provided written consent.

RESULTS

Clinical characteristics of patients and reactions

Between 2009 and 2014, 111 subjects with AERD underwent aspirin desensitizations at our institution and agreed to participate in clinical research. Of these, 29 subjects provided urine for eicosanoid analysis, and 11 provided blood for complete blood count before desensitization and after 2 months of aspirin treatment. 23 (Group I) completed the desensitization and successfully initiated treatment with high-dose aspirin, and 6 (Group II) were unable to tolerate the desensitization due to marked extrapulmonary symptoms (*n*=5) or failure of lower respiratory tract symptoms to resolve (*n*=1). Age, race, baseline FEV₁ (% predicted), FEV₁/FVC ratio, ICS use, total IgE and peripheral blood eosinophil counts were not statistically different between the groups (Table I). Use of ICS or oral steroids remained the same throughout the study.

Subjects in both AERD Group I and Group II experienced reductions in their FEV₁ ($15 \pm 3\%$ for Group I, $30 \pm 10\%$ for Group II, *P* < .05, Table II) during reactions to aspirin. The provocative aspirin dose (log₂) that triggered the reaction was not significantly different between the two groups (Table II) and did not correlate with PG metabolite levels. Abdominal pain during reaction was more common (*P* < .05) and more severe in Group II with 4 subjects reporting sharp stabbing pain, 3 reporting nausea, and 1 subject experiencing watery diarrhea. A rash appeared during the reactions in all subjects in Group II, while none of the subjects in Group I developed rash (*P* < .001). The rash consisted of an erythematous and pruritic macular eruption that began on the distal upper and lower extremities bilaterally. It often involved the palmar and plantar surfaces (Fig 1), spread proximally and was not associated with urticaria or angioedema. Five of the 6 subjects in Group II were unable to tolerate aspirin therapy with dose escalation due to the persistence of gastrointestinal symptoms and/or rash. The one subject in Group II who eventually was able to tolerate aspirin therapy required a slower desensitization spaced over 2 days but continued to have rash and lower respiratory reactions with each successive dose of aspirin.

Urinary eicosanoid measurements and relationship to clinical outcomes

Basal levels of urinary LTE_4 were higher in AERD Group II (2.9 ± 1.0 pmol/mg Cr) than in the ATA control group (0.2 ± 0.09 pmol/mg Cr, $P < .01$, Fig 2). Compared to their respective pre-reaction basal levels, the urinary levels of LTE_4 in both AERD groups increased during the reaction to aspirin (Group I 10.5 ± 1.8 , Group II 16.5 ± 4.6 pmol/mg Cr, $P < .05$ for both). Peak LTE_4 levels were not significantly different.

PGE-M was the most abundant prostanoid metabolite detected at baseline in all three groups (Fig 3A, left panel). The basal levels of PGD-M ($P < .05$) and TX-M ($P < .01$) were higher in the Group II (13.6 ± 2.7 and 1.4 ± 0.3 pmol/mg Cr, respectively) than the Group I (7.0 ± 0.8 and 0.9 ± 0.1 pmol/mg Cr, respectively). Total basal urinary PG metabolites in Group II subjects exceeded that in Group I subjects by ~2-fold (43 ± 2 vs. 23 ± 2 pmol/mg Cr, $P < .01$, not shown).

Following the provocative dose, urinary PGD-M increased in 8, decreased in 12, and remained unchanged in 3 of the 23 subjects in Group I, but increased in all 6 subjects in Group II. As a result, the level of PGD-M during the reaction was ~7-fold higher in the urine of Group II subjects than Group I subjects (61.3 ± 19.9 vs. 9.6 ± 1.8 pmol/mg Cr, respectively, Fig 3A, right panel, $P < .001$). Those 8 subjects in Group I who showed increased PGD-M during reaction had a basal level of 5.6 ± 0.6 which increased to 18.2 ± 3.2 pmol/mg Cr. The levels of PGI-M (0.7 ± 0.2 vs. 0.3 ± 0.05 pmol/mg Cr; $P < .001$) and TX-M (2.4 ± 0.7 vs. 0.7 ± 0.1 pmol/mg Cr; $P < .001$) during the reaction were significantly higher in the post-aspirin urine of Group II than Group I (Fig 3A).

Total PG metabolites in the urine of Group II subjects were significantly greater than total metabolites in the urine of the Group I subjects during the reaction (85 ± 22 vs. 21 ± 3 pmol/mg Cr, $P < .001$, not shown). All PG metabolites decreased in the urine of the ATA controls ($n=5$, data not shown) after the ingestion of 325 mg of aspirin with the exception of one subject who had a rise in PGD_2 . Figure 3B shows the $\log_2$ of the fold change (post-aspirin compared to basal level) of each metabolite in all three subject groups.

Basal and reaction eicosanoids were assessed for correlation with fall in FEV_1 during aspirin reaction. Both basal urinary LTE_4 ($r = -.461$, $P < .05$) and PGD-M ($r = -.438$, $P < .02$) inversely correlate with maximum fall in FEV_1 during the reaction (Fig 4, AB). Reaction PGD-M ($\log_2$) also correlated with fall in FEV_1 during reaction ($r = -.41$, $P < .05$, not shown).

To determine the effect of high-dose aspirin therapy on the production of eicosanoids in subjects with AERD, we compared basal levels of urinary PG metabolites and LTE_4 to levels after 8 weeks of high-dose aspirin therapy in Group I subjects ($n=14$). Aspirin therapy resulted in significant decreases in PGE-M (8.7 ± 2.1 pmol/mg Cr, $P < .01$), PGI-M (0.14 ± 0.03 pmol/mg Cr, $P < .01$), TX-M (0.2 ± 0.1 pmol/mg Cr, $P < .001$) and PGD-M (2.2 ± 0.8 pmol/mg Cr, $P < .001$) from basal levels, but there was no change in LTE_4 levels (2.7 ± 1.2 pmol/mg Cr, $P = .24$, Fig 5). The one subject in Group II who went on to tolerate high-dose aspirin also demonstrated a fall in PGD-M from baseline after 8 weeks.

Peripheral blood leukocyte counts and relationship to urinary PGD-M

Absolute peripheral blood eosinophil, basophil, and neutrophil counts were assessed in Group I AERD subjects ($n=11$) at baseline and again on high-dose aspirin. While on high-dose aspirin therapy, eosinophil counts rose in all subjects (0.31 ± 0.06 to 0.93 ± 0.19 K/ μ L, $P<.01$), and basophil counts rose or stayed the same (0.06 ± 0.02 to 0.09 ± 0.02 K/ μ L, $P=.08$). Neutrophil counts did not change (Fig 6). The percent increase in eosinophils on high-dose aspirin therapy trended to inversely correlate to the percent fall in urinary PGD-M from basal levels on high-dose aspirin ($r=-0.51$, $P=.16$; data not shown). The one subject in Group II who went on to tolerate high-dose aspirin also demonstrated an increase in absolute peripheral blood eosinophils at 8 weeks.

Discussion

AERD involves complex dysregulation of proinflammatory (cysLTs, PGD₂) and anti-inflammatory eicosanoid mediators (PGE₂). Clinical reactions to drugs that block COX-1 are characterized by increases in urinary LTE₄ and have been thought to be largely driven by the effector functions of cysLTs³². To our knowledge, ours is the first comprehensive examination of PG generation in subjects with AERD undergoing a therapeutic desensitization procedure. Unexpectedly, subjects unable to tolerate the procedure due to cutaneous and gastrointestinal symptoms (despite the use of a CysLT₁ receptor antagonist) not only generate higher levels of cysLTs than those who tolerate the procedure, but also fail to suppress several prostanoids at a threshold dose of aspirin. In particular, individuals unable to tolerate the procedure generate markedly more PGD₂ during reactions than do those who tolerate desensitization. These findings imply a potential role for “aspirin resistant” PGD₂ as a mediator of AERD. Additionally, the suppression of PGD₂ that results from high-dose aspirin therapy may prevent the recruitment of PGD₂-responsive effector cells to the target tissue. This could account for why therapy with high-dose aspirin improves disease control without altering the high baseline urinary LTE₄ levels typical of this disease^{24,33, 34}, while increasing the blood levels of eosinophils and basophils.

The demographic and physiologic characteristics of the 23 subjects who tolerated the desensitization procedure (Group I) were comparable to those who did not tolerate the procedure (Group II) (Table 1). Every subject in Group I developed respiratory symptoms during the procedure, but these resolved and all subjects successfully continued on to higher aspirin doses without additional symptoms. The subjects in Group II also developed greater bronchoconstriction (Table 2), but were unable to complete the procedure due to ongoing abdominal pain and/or an atypical rash (Fig 1). The development of a rash during desensitization signaled a more challenging desensitization for subjects. The gastrointestinal manifestations could well reflect the actions of mediators released from gut mast cells during the reaction to aspirin³⁵. The dose of aspirin that provoked symptoms was not statistically different between the groups (Table 2) and did not correlate with reaction PGD-M, consistent with previous studies reporting that neither the severity of reactions nor the changes in urinary LTE₄ depend on the dose of aspirin ingested^{36,13}. We cannot exclude the possibility that the subjects in Group II might tolerate an alternative protocol using

intranasal ketorolac, which may be better tolerated than the standard oral aspirin desensitization protocol used in our study³⁷.

The correlation between basal LTE₄ and the fall in FEV₁ (Fig 4,A) is consistent with the observations of Daffern et al, who reported that baseline urinary LTE₄ levels predict the severity of airflow obstruction during aspirin challenges¹³. Unlike the Daffern study, however, all but two subjects in our cohort were pretreated with a CysLT₁ receptor antagonist. Thus, the fact that basal urinary levels of LTE₄ correlated with the fall in FEV₁ in our subjects suggests that the cysLT-dependent component of airflow obstruction involves receptors other than CysLT₁ receptors. Since we found that PGD-M levels at baseline and during reaction also correlated with the decrease in FEV₁ (Fig 4,B), the bronchoconstricting effects of PGD₂ could account for the portion of the bronchial response to aspirin that resists CysLT₁ receptor blockade.

Although previous studies have monitored the production of PGs during aspirin challenges in AERD, none had simultaneously monitored all PG metabolites in subjects with vastly different clinical responses to aspirin. PGE₂ suppresses 5-LO activity³⁸. PGD₂ and TXA₂ are potent bronchoconstrictors³⁹, and PGI₂ is a vasodilator that decreases airflow when inhaled⁴⁰. Thus all PGs measured in this study are potentially relevant to AERD. The increase in total PG metabolites observed in Group II during reactions to aspirin (from 43.2±9.4 to 85.2±22.1 pmol/mg Cr) contrast with the aspirin-induced changes in PGs observed in Group I and in the ATA controls. While much of the increase in total PGs in Group II reflects PGD-M, TX-M and PGI-M levels tended to increase in response to aspirin challenge in this group, whereas both tended to decrease in Group I (Fig 3, A,B). Although PGE₂ is bronchoprotective in AERD due to inhibition of mast cell activation and 5-LO, PGE-M levels did not change significantly in either group during reactions to aspirin. It is possible that the high levels of PGE₂ generated by the kidney mask changes that reflect aspirin-induced suppression of its production in the respiratory tissue^{41,42} at the low doses that elicit reactions. The slight reduction in PGE-M observed in Group I after 8 weeks of treatment may reflect the ability of high dose aspirin to interfere with COX-2 (from which most renal PGE₂ is derived) as well as COX-1. The fact that both LTE₄ levels and PG levels rose simultaneously suggests that aspirin provokes the release of large quantities of AA in Group II subjects by a to-be-determined mechanism. Additionally, the comparative resistance of their PG metabolites to suppression by aspirin at doses that block COX-1 (but not COX-2) suggests that COX-2 activity may mediate PG production in Group II subjects⁴³. COX-2 protein expression by mast cells is increased in bronchial biopsies from patients with AERD compared to ATA controls⁴⁴. COX-2 selective antagonists are almost universally well-tolerated by patients with AERD⁴⁵, but whether COX-2 antagonists might actually be *therapeutic* in Group II AERD subjects remains to be determined.

As the effects of high-dose aspirin therapy on PG metabolites had not previously been studied comprehensively in AERD, we sought to determine the effect of high dose-aspirin therapy on urinary eicosanoids in Group I subjects. The sharply reduced levels of PGI-M, TX-M and PGD-M in the urine of Group I subjects while on aspirin (Fig 5) suggest that suppression of these bronchoconstrictive PGs may contribute to the clinical benefit of high-dose aspirin therapy.

PGD₂ is potently chemotactic for eosinophils and basophils, both of which express CRTH2/DP2²¹. Aspirin challenges induce a reduction in blood eosinophil counts in subjects with AERD², potentially reflecting (in retrospect) their recruitment to the tissue. Both eosinophils and basophils increase in nasal lavage fluids following aspirin challenge¹, without an accompanying increase in the concentrations of eosinophil-active chemokines. Remarkably, the numbers of eosinophils rose, but not neutrophils (which lack CRTH2/DP2) (Fig 6), in all successfully desensitized subjects in our study, and tended to correlate with the reduction in PGD-M. We suspect that PGD₂, through CRTH2/DP2, may facilitate the persistent eosinophilic inflammation of the respiratory tract that characterizes AERD. The suppression of PGD₂ generation by high-dose aspirin therapy may increase the circulating pool of CRTH2/DP2⁺ effector cells by removing the chemotactic gradient that supports their recruitment to the tissue. Suppression of effector cell recruitment could contribute to the therapeutic benefit of high-dose aspirin in AERD, which is clearly not due to changes in the production of cysLTs⁴⁶.

Previous studies had reported that levels of PGD₂ metabolites in urine, plasma, or BAL fluid either remain unchanged^{24,33,47,4}, or increase modestly with aspirin challenges in subjects with AERD⁴⁸. The differences between these and our data likely reflect differences in sampling, the heterogeneous nature of AERD⁴⁹, and the variable proportions of subjects with the phenotype defined by Group II. The pharmacologic properties of PGD₂ and its metabolites make it likely that they play a causal role in the severe extrapulmonary manifestations of reactions to aspirin in Group II. The significant correlation between basal urinary PGD-M levels and the fall in FEV₁ during aspirin reaction suggest that they also contribute to the respiratory response to aspirin (Fig 4). PGD₂-mediated bronchoconstriction is sensitive to blockade with antagonists of the thromboxane receptor^{48,50}. PGD₂ also causes cutaneous vasodilation by acting at DP₁ receptors²¹, and it seems plausible that the rash experienced by subjects in Group II (Fig 1) could reflect the direct effects of PGD₂ in the skin microvasculature. Finally, the selective changes in blood eosinophils and basophils resulting from high-dose aspirin therapy in our study (Fig 6) may well reflect a prominent function for PGD₂ in recruiting CRTH2/DP2⁺ effector cells to the respiratory tissues. Drugs are under development that block thromboxane, DP₁, and DP₂/CRTH2 receptors^{50,51}. We speculate that these drugs, alone or in combination, could permit safe desensitization to aspirin in the PGD₂-overproducing subgroup of patients with AERD, and could replicate some of the therapeutic benefits of high-dose aspirin by preventing the chemotaxis of CRTH2/DP2⁺ cells into the tissues. Our study is a necessary prerequisite to such trials.

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Abbreviations

AERD	Aspirin exacerbated respiratory disease
ASA	Aspirin

COX-1	cyclooxygenase-1
COX-2	cyclooxygenase-2
CysLT₁	type 1 receptor for the CysLTs
DP	D prostanoid
PG	prostaglandin
PGD₂	Prostaglandin D ₂
PGE₂	Prostaglandin E ₂
LTE₄	Leukotriene E ₄
CysLT	Cysteinyl leukotrienes
TXA₂	Thromboxane
PGI₂	Prostacyclin
PGI-M	Prostacyclin metabolites
PGD-M	Prostaglandin D metabolites
PGE-M	Prostaglandin E metabolites
TX-M	Thromboxane metabolites
5-LO	5-lipoxygenase
ATA	aspirin-tolerant asthmatics
AA	Arachidonic acid
BAL	bronchoalveolar lavage
DP₂/CRTH2	D prostanoid receptor 2/chemokine receptor homologous molecule expressed on Th2 lymphocytes

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Key Messages

- Cutaneous and gastrointestinal symptoms during aspirin desensitization and failure to tolerate desensitization in subjects with AERD are associated with marked increases in PGD₂ generation.
- High-dose aspirin therapy in subjects with AERD suppresses PGD₂ production and raises peripheral blood eosinophil counts.

Capsule summary

- Increased PGD₂ generation is associated with severe extrapulmonary reactions during aspirin desensitization in subjects with AERD. High-dose aspirin therapy suppresses PGD₂ production, which may prevent the recruitment of effector cells into the respiratory tissue.

Figure 1.
Rash observed following provocative dose of aspirin in 3 Group II AERD patients.

Figure 2. Basal and post-aspirin (ASA) urinary LTE₄ levels

Basal and aspirin-induced urinary LTE₄ levels analyzed by GC-MS from ATA controls (basal n=10, post-aspirin n=5), Group I AERD subjects (n=23) and Group II AERD subjects (n=6) are shown. Data are expressed as mean +SEM (**P*<.05, ***P*<.01).

Figure 3. Basal and post-aspirin (ASA) PG levels

(A) Basal (left panel) and post-aspirin administration (right panel) urinary PG levels in subjects with ATA (basal n=10, post-aspirin n=5), Group I (n=23) and Group II (n=6) are shown. (B) Log₂ of change from basal levels in urinary PGs induced by aspirin administration is shown for the three patient groups. Data are expressed as mean +SEM (* $P < .05$, ** $P < .01$, *** $P < .001$).

Figure 4. Basal urinary eicosanoid levels correlate with change in FEV₁ during aspirin desensitization

Basal urinary (A) LTE₄ and (B) PGD-M levels plotted against the corresponding percent change in FEV₁ during aspirin reaction for each subject with AERD. Effect size, determined with Pearson correlation coefficient, is denoted as an r value.

Figure 5. Effect of high-dose aspirin therapy on urinary eicosanoid levels in AERD

Urinary eicosanoid levels of Group I AERD subjects (n=14) are shown at baseline pre-aspirin and after 650mg twice-daily aspirin therapy for 8 weeks. Data are expressed as mean +SEM (** $P < .01$, *** $P < .001$).

Figure 6. Peripheral blood eosinophils increase on high-dose aspirin therapy

Blood eosinophil, basophil, and neutrophil counts (K/ μ L) for Group I AERD subjects (n=11) at baseline and on high-dose aspirin. Data are expressed as mean $\pm$ SEM (** $P < .01$).

Table I

Baseline clinical characteristics according to group stratification.

Clinical Characteristics	Aspirin-Tolerant Asthmatics (n=10)		Group I AERD (n=23)		Group II AERD (n=6)		P Value (Group I vs. Group II)
	mean	SEM	mean	SEM	mean	SEM	
Age (years)	36.3	±3.3	47.3	±2.3	47.3	±1.7	.99
Male	40%		39%		50%		.67
Baseline FEV1 (% predicted)	91%	±6	84%	±3	86%	±4	.63
FEV1/FVC	80%	±3	73%	±2	71%	±2	.87
Total IgE (IU/mL)			168.0	±35	344.4	±85	.22
Peripheral Blood Eosinophils (k/μL)			0.66	±0.11	0.40	±0.20	.16
ICS + LABA	90%		91%		100%		.33
High	0%		9%		17%		
Medium	10%		57%		50%		
Oral Prednisone	0%		13%		17%		1.0
Montelukast Use	40%		91%		100%		1.0

Data are displayed as mean ± standard error of the mean (SEM). FEV₁ – forced expiratory volume; ICS - inhaled corticosteroid; LABA – long-acting beta agonist.

Table II

Clinical characteristics of AERD subjects stratified by group following the provocative dose of aspirin.

Clinical Characteristics During Aspirin Desensitization					
	Group I AERD (n=23)		Group II AERD (n=6)		P Value
	mean	SEM	mean	SEM	
Maximum Change in FEV ₁	-15%	±3	-30%	±10	<.05
Abdominal Symptoms	26%		83%		<.05
Rash	0%		100%		<0.001
Provocative Aspirin Dose (mg)	113	±16	68	±30	0.18

Data are displayed as mean ± standard error of the mean (SEM). FEV₁ – forced expiratory volume.