

Pathogenesis of Aspirin-Exacerbated Respiratory Disease

Donald D. Stevenson,^{*,1} and Bruce L. Zuraw²

¹Allergy and Immunology Division, Scripps Clinic and The Scripps Research Institute, La Jolla, CA 92037; ²Department of Experimental and Molecular Medicine, The Scripps Clinic and Research Institute, La Jolla, CA 92037

Abstract

The underlying respiratory disease is activated by unknown mechanism and results in an intense infiltration of mast cells and eosinophils into the entire respiratory mucosa. These cells synthesize leukotrienes (LTs) at a very high rate and mast cells also release histamine and tryptase and synthesize PGD₂ a vasodilator and bronchoconstrictor. Furthermore, AERD patients synthesize from arachidonic acid (AA) a peculiar product called lipoxins, which opposes inflammation generated by leukotrienes. Finally, cysLT₁ receptors are over expressed and highly responsive to LTE₄, further augmenting the underlying inflammatory disease.

This inflammatory condition is partly inhibited by synthesis of PGE₂ through COX-1. PGE₂ partially inhibits 5-lipoxygenase conversion of AA to LTA₄ and blocks release of histamine and tryptase from mast cells. When COX-1 is inhibited by ASA or NSAIDs, PGE₂ synthesis stops and an enormous release of histamine and synthesis of LTs occurs. The upper respiratory reaction is mediated by both histamine and LTs but the bronchospastic reaction is mediated by LTs. The systemic effects of flush, gastric pain and hives are mediated by histamine.

Aspirin desensitization can not be explained by disappearance of LT synthesis since urine LTE₄ levels are still elevated at acute ASA desensitization. However, mast cell products such as histamine, tryptase and PGD₂ are no longer released or synthesized at acute desensitization. It is more likely that a diminution in number or function of cysLT receptors accounts for the diminished inflammatory response found in ASA desensitization.

Index Entries:

Aspirin-exacerbated respiratory disease (AERD); leukotrienes (LTs); leukotriene E₄ (LTE₄); leukotriene A₄ (LTA₄); Cysteinyl leukotriene receptors (cysLTR); prostaglandin E₂ (PGE₂); prostaglandin G₂ (PGD₂); arachidonic acid (AA).

*Author to whom all correspondence and reprint requests should be addressed. E-mail: dstevemd@aol.com

Introduction

The clinical presentation of reactions to ASA and other NSAIDs is compatible with both allergic and pseudoallergic reactions. Although difficult to definitively establish, immediate hypersensitivity reactions (urticaria, angioedema, anaphylaxis) to single drugs in this class are most likely IgE-recognized and -mediated. By contrast, first-exposure reactions to chemically dissimilar NSAIDs have been established as pseudoallergic reactions, which rely upon shared actions of pharmacologic inhibition of cyclooxygenase enzymes.

In 1971, Vane (1) discovered that both ASA and NSAIDs inhibit prostaglandin synthase, now renamed cyclo-oxygenase (COX). Recent findings have identified at least two isoforms of COX: COX-1 is constitutively expressed in most mammalian cells, including respiratory and gut epithelial cells and most inflammatory cells; and COX-2, which is 60% homologous with COX-1, and is highly inducible by pro-inflammatory mediators, such as cytokines, growth factors, and physiologic tissue injury (2). ASA and most NSAIDs inhibit both COX-1 and 2, although they are far more potent inhibitors of COX-1. By contrast, the new selective COX-2 inhibitors are potent and specific inhibitors of the COX-2 enzyme, with no measurable effect on COX-1 in the doses used in human pharmacology (3). Whether COX-1 and or COX-2 are genetically different from normals has not been determined. However, this critical enzyme system is the key to crossreactions, and may be phenotypically different in aspirin-exacerbated respiratory disease (AERD) patients.

In 1980, Samuelsson reported that a second metabolic pathway for arachidonic acid substrate, namely the 5-lipoxygenase (5-LO) pathway, where leukotrienes are synthesized, was also involved in the genesis of inflammation (4). Leukotrienes (C,D,E₄) are potent mediators of the chemotaxis of eosinophils, increased vascular permeability, mucus gland secretion, and prolonged and powerful constriction of bron-

chial smooth muscles (5). Since the discoveries of Vane and Samuelson, research has advanced our knowledge about the genetic control of inflammatory enzyme systems, recruitment and movement of inflammatory cells, and the mechanisms by which ASA/NSAIDs inhibit or form arachidonic acid products within these cells (6).

Pathogenesis of the Underlying Respiratory Disease

Inflammatory Cells

Mast cells and eosinophils have been identified in nasal cytograms and nasal-tissue biopsies from AERD asthmatics (7). In a study by Yamashita et al. (8), immunohistochemical staining of polyp tissues from AERD patients revealed numerous eosinophils and degranulated mast cells. In AERD asthmatics, in contrast to ASA-tolerant asthmatics, bronchial biopsies contained increased numbers of eosinophils and mast cells, which stained for 5-LO (9), COX-2 (10), interleukin-5 (IL-5) and granulocyte-macrophage-stimulating factor (GM-CSF) (11). After stimulation with ASA-lysine, bronchoalveolar lavage (BAL) fluid from asthmatic AERD subjects contained increased numbers of eosinophils as well as increased levels of eosinophil cationic protein (ECP) in AERD asthmatics, compared to control asthmatics (12). Similar results were obtained by Kowalski et al. (13) while conducting nasal lavages in AERD patients after instilling ASA-lysine into the noses of six ASA-sensitive asthmatic volunteers. Significantly increased numbers of eosinophils were found, when compared to normals and patients with chronic allergic rhinitis. Although metachromatic cells increased (21/mm³ to 64 cells/mm³), the difference was not statistically significant in this small study population. Leukocytes (PMNs) and macrophages are found in respiratory tissues and secretions of both AERD patients and ASA-tolerant asthmatics (9,12). Since these inflammatory cells also synthesize arachidonate products,

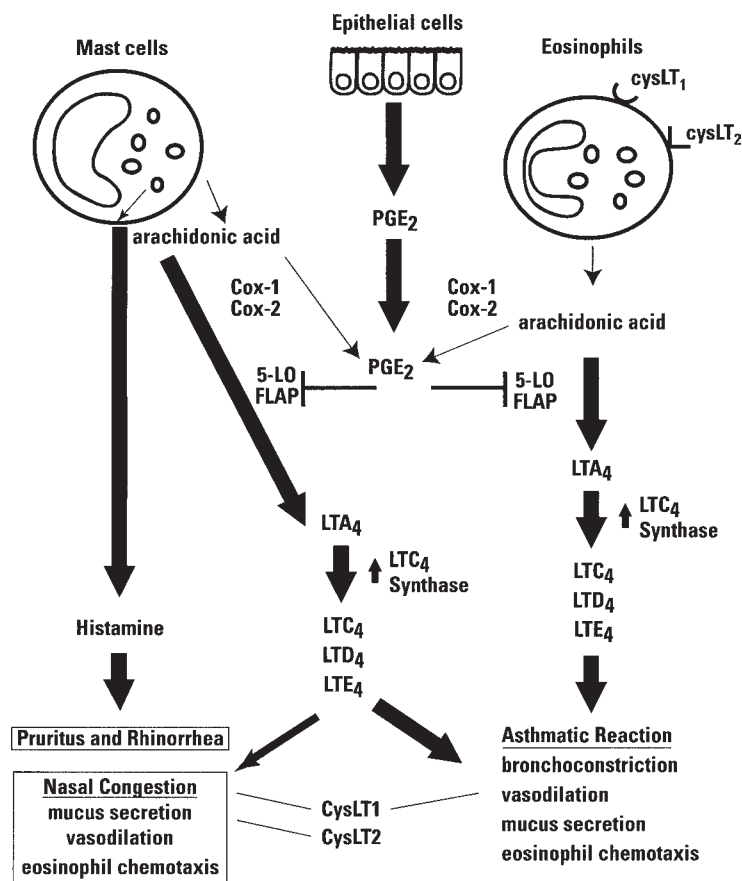


Fig 1. Mast cells and eosinophils heavily infiltrate the submucosal surface of the respiratory epithelium in AERD. All three cells synthesize prostanoids, including PGE₂ via COX-1. Mast cells and eosinophils also synthesize leukotrienes (LTs) and, through upregulated COX-2, synthesize both inflammatory and inhibitory prostanoids. Mast cells and eosinophils also secrete preformed mediators. In aspirin disease, the inflammatory cascades are active and only partially downregulated by PGE₂. When ASA or NSAIDs are ingested, the PGE₂ brake is removed and an avalanche of mediators induces a marked increase in the pathophysiologic events shown at the bottom of the figure. CysLT₁ and ₂ receptors are present in the upper airway, but only CysLT₁ receptors are found on bronchial smooth-muscle cells. During ASA desensitization, both COX-1 and -2 are destroyed and histamine and eosinophil cationic protein (ECP) and perhaps other eosinophil products are no longer released. LTs are synthesized, but at a rate no greater than baseline. CysLT₁ receptors, and by speculation CysLT₂ receptors, appear to be less responsive or available to the stimulus of LTs.

their potential for participation in the inflammatory disease of AERD cannot be dismissed. However, a preferential accumulation of these cells in the respiratory mucosa, as is seen with mast cells and eosinophils, has not been reported in AERD patients (see Fig. 1).

Chronic Hyperplastic Eosinophilic Sinusitis, Nasal Polyps, and Asthma

The hallmark of this condition is hyperplastic mucosal inflammation and nasal polyps

(14). It is found in virtually all AERD patients, who are generally recognized to be the prototype example of the syndrome of chronic hyperplastic eosinophilic sinusitis. This inflammatory mucosal disease is characterized by heavy infiltration of eosinophils, and is associated with the activation of TH₂ lymphocytes, producing IL-3, IL-4, IL-5, IL-13, eotaxin, and GM-CSF (14–16). Furthermore, infiltrating eosinophils contribute additional cytokines—with IL-5, for example—recruiting more eosinophils to the

respiratory mucosa, which is already infiltrated by activated eosinophils and mast cells. Thus, the cells that are infiltrating nasal, sinus, and bronchial mucosa are also capable of prolonging inflammation by recruiting more eosinophils to join the inflammatory process. Using nasal polyp tissue, Kowalski et al. (17) demonstrated that apoptosis of eosinophils was significantly delayed in AERD patients, when compared to ASA-tolerant controls. Cytokines such as IL-3, IL-5, and GM-CSF dramatically increased the lifespan of eosinophils in vitro by inhibition of programmed cell death (18). Thus, delayed cell death of eosinophils guarantees both infiltration and survival of eosinophils in the respiratory mucosa of AERD patients. Furthermore, this heavy infiltration of activated eosinophils in the respiratory mucosa is also responsible for further tissue damage, activation of fibroblasts, and thickening of basement membranes (14).

Nasal polyps consist of edematous tissue infiltrated by eosinophils. Extensive studies have shown that the prevalence of nasal polyps is considerably higher in nonallergic rhinitis, than in allergic rhinitis, but all patients with nasal polyps also suffer from AERD (19). However, in AERD, it is unusual to find patients without nasal polyps (20,21). The histology of nasal polyps consists of edematous and fibrotic stroma, reduced numbers of vessels and glands, and no neural structures, surrounded by a thickened basement membrane and a damaged epithelium (22). Eosinophils are found throughout the polyps, but are particularly prominent between the epithelium and thickened basement membrane. Mediators in normal nasal mucosa vs nasal polyp-tissue supernatants have been measured. A highly significant increase in IL-5, eotaxin, eosinophilic cationic protein, and albumin in the supernatants of non-allergic nasal polyps was found, but there was no difference in concentrations of leukotrienes C4-E4 or hyaluronic acid (23). Eotaxin is a chemokine, which is important in recruitment and activation of eosinophils and may

also contribute to tissue damage through its capacity to induce the release of reactive oxygen radicals (24). Mullol et al. (25) used cultured cells from aspirin-tolerant nasal polyps and normal nasal mucosal cells, and showed a significant decrease in COX-1 mRNA. Furthermore, after stimulation by cytokines, there was a delay in synthesis of COX-2 mRNA in the nasal polyp cells vs normal mucosal cells (4 h vs 1 h). This finding is very interesting because it implies that polyp cells do not synthesize the protective prostanoid (prostaglandin E₂, or PGE₂), and that this finding is not limited to patients with AERD. Bachert and his colleagues (23) have proposed a mechanism for eosinophilic polyp formation. Accumulation and activation of eosinophils in mucosal tissue, particularly under the direction of IL-5 and eotaxin, characterizes the first stage of polyp formation. Activated eosinophils release vasoactive and cytotoxic substances (ECP, MBP, EPO, and EDN), increasing vascular permeability and damaging mucosal tissue. Exudation of albumin and fibronectin and retention of water occur next. Pseudocysts form, and eosinophils, mast cells, and fibroblast infiltrate the polyp stroma. Gravitational pull enhances polyp elongation. Activation of myofibroblasts by these mediators contributes to the development of chronic fibrosis.

Leukotrienes and Prostanoids

There is clear evidence that AERD patients constitutively synthesize excessive amounts of archidonate products, even before any exposure to ASA/NSAIDs (see Figs. 1, 2). For example, at baseline, prior to challenge with ASA-lysine, Sladek et al. (12) measured higher concentrations of LTC₄ and TXB₂ in BAL, taken from AERD asthmatics than from control asthmatics and normals. When compared to normal controls and ASA-tolerant asthmatics, Christie and colleagues (26) and Smith et al. (27) reported elevated levels of LTE₄ and TXB₂ in the urine of ASA-sensitive asthmatic patients prior to ASA challenge. Since LTE₄ is the terminal leukotriene

in the synthesis of leukotrienes, and is synthesized in extracellular fluid, urinary concentrations of LTE_4 reflect prior synthesis of leukotrienes in both the upper and lower respiratory tracts or elsewhere in the body. High levels of urinary TXB_2 are the consequence of excess synthesis of COX products. Subsequent studies have consistently corroborated the *in vivo* evidence of increased synthesis of leukotrienes and prostanoids in most AERD asthmatics, before any exposure to ASA (28–31). Certainly, eosinophils and mast cells are capable of synthesizing cysteinyl leukotrienes (32–35) and are likely to be primary cells generating these mediators in the respiratory mucosa of AERD patients.

The presence of both LTC_4 and eosinophil cationic protein in BAL fluid during ASA challenges establishes not only that eosinophils are present in the bronchial airways, and that they are also activated and releasing mediators (36). Eosinophils generate leukotrienes and also express $\text{cysLT}_{1\text{ and }2}$ receptors. In the presence of GM-CSF, IL-3, and IL-5, leukotrienes stimulate proliferation and differentiation of eosinophil progenitor cells in the bone marrow (37). Leukotrienes are also chemotactic for eosinophils, directing blood-borne eosinophils to the respiratory mucosa (38). Mast cells in the respiratory tract can also synthesize large amounts of LTC_4 , D_4 , and E_4 as well as prostaglandin D_2 (39). Therefore, the evidence indicates that eosinophils and mast cells (34), are a major source of leukotrienes in the respiratory mucosa. However, it is impossible to estimate the relative production of leukotrienes by other cells in the airways capable of synthesizing leukotrienes, including monocytes (40), polymorphonuclear leukocytes (41), and basophils. Furthermore, prostanoids are also synthesized by epithelial cells of the airways (42) and endothelial cells of the supporting vessels (43–45).

CysLT-Receptors

In recent years, two distinct leukotriene receptors have been indentified, and both are activated by binding of LTC_4 , D_4 , or E_4 . CysLT_1 was discovered and cloned by two independent groups in 1999 (46,47). In normal subjects, cysLT_1 receptors are found on bronchial smooth muscle, endothelial cells, mucus-secreting glands, as well as mature and progenitor eosinophils, lung macrophages, and subsets of peripheral-blood monocytes (CD, 19, 34, and 14) and T cells (CD4, CD8) (48). The cysLT_2 receptor was discovered in 2000 (49). It is present on eosinophils, neutrophils, monocytes, and T lymphocytes, as well as endothelial cells and Purkinje fibers in the heart (50). Mita and colleagues (51) found that peripheral blood leukocytes from asthmatics express cysLT_2 receptors and that eosinophils express twice as much mRNA for cysLT_2 as cysLT_1 . The implication of this observation is that selectively blocking cysLT_1 receptors may not significantly prevent leukotriene effects on eosinophils. James and Sampson (50) further suggested that there may be a third cysLT receptor, which to date has not been discovered, and it also would not be blocked by cysLT_1 -receptor antagonists. Whether or not such a receptor exists is not yet known.

Strong circumstantial evidence supports an important role for leukotriene receptors in the pathophysiology of AERD. Because only cysLT_1 is expressed on bronchial smooth muscle, its function is unique in inducing smooth-muscle constriction of the bronchial airways. AERD subjects have been shown to be specifically hypersensitive to the bronchoconstrictive effect of inhalation of LTE_4 ; and selective cysLT_1 -receptor antagonists can block ASA-induced bronchoconstriction in some AERD patients, consistent with the lack of expression of cysLT_2 receptors in bronchial smooth muscle (52–54). The presence of both cysLT_1 and cysLT_2 receptors in the upper airway mucosa, including endothelial cells, mucus-secreting glands, T-lymphocytes, polymorpholeukocytes, and eosinophils, makes it likely that stimulation of

cysLT₂ receptors also contributes to the chronic eosinophilic upper-airway inflammation seen in AERD patients. These observations are supported by the fact that cysLT₁-receptor antagonists are somewhat effective in blocking smooth-muscle constriction in the lower airways, but usually have only partial or no effects on nasal congestion and rhinorrhea in AERD patients (55). Although it is theoretically possible that cysLT₁- and cysLT₂-receptor expression is quantitatively or qualitatively different in AERD patients, the expression of these receptors has not been quantified in the airway tissues of AERD patients. It is also theoretically possible that leukotriene receptors transduce their signals more efficiently in AERD patients, thereby augmenting the effects of cysLTs and potentially producing a more vigorous inflammatory response. For example, there is some evidence that within the AERD population, different degrees of responsiveness to stimulation of one receptor over the other may be occurring, depending on genetic differences in the transcription and expression of these receptors. Clinically, some AERD patients enjoy marked decongestion of their nasal tissues, remissions in asthma, and marked increases in their forced expiratory volume in one second (FEV₁) values, while taking a cysLT₁-receptor antagonist. Other AERD patients who are clinically indistinguishable from these patients may not receive a benefit from taking a cysLT₁-receptor antagonist. Without availability of a selective cysLT₂ antagonist, this discrepancy in responses to currently available cysLT₁-receptor antagonists cannot be further explored. Unfortunately, the prospects for developing selective cysLT₂-receptor antagonists may not be feasible because of potential side effects of such drugs on the regulation of Purkinje fibers in the heart (56) or cysLT₂ receptors in other tissues.

Lipoxins

In addition to generating increased leukotrienes and prostanoids, AERD patients may also generate less of the antiinflammatory lipid

mediators, lipoxin and 15-epimer lipoxin (see Fig. 2). Like leukotrienes, lipoxins are synthesized from arachidonic acid by lipoxygenase enzymes. However, their function is the opposite of the pro-inflammatory leukotrienes because they downregulate inflammation (57). In a study by Sanak et al. (58), AERD patients generated less lipoxins and 15-epimer lipoxins following stimulation of blood cells with calcium ionophore than did ASA-tolerant asthmatics and normal subjects. Furthermore, pretreatment of blood cells with aspirin led to increased synthesis of lipoxins in the cells of ASA-tolerant asthmatics, but not cells from AERD patients. This suggested that the lipoxins, which were designed to dampen and limit inflammation, are preferentially undersynthesized in AERD patients. Whether this is the central defect in AERD and is responsible for uncontrolled leukotriene synthesis and mucosal inflammation, or merely one dysfunction with perhaps little or no consequence, cannot be determined at this time.

Initiating and Propagating Stimuli in Producing AERD

Unfortunately, the initiating and propagating stimuli in AERD are currently unknown. It is as though we have been allowed to examine the consequences of a fundamental stimulatory event—namely, uncontrolled synthesis of arachidonate products by infiltrating mast cells and eosinophils—without being able to see why this process began in the first place. Since the inflammatory disease of AERD is similar to chronic allergic rhinitis and asthma, with infiltrating eosinophils and mast cells, AERD could hypothetically be caused by chronic allergen exposure with recognition by IgE antibodies. However, there is no convincing evidence that IgE recognition and mediation plays a role in the initiating or propagating events in AERD. That is not to say that IgE-mediated respiratory disease cannot coexist with AERD. In fact, 30–60% of patients with AERD have positive wheal-and-flare skin tests and histories that support

IgE-mediated respiratory events (59,60). It is not rare, to evaluated AERD patients who give a history of sneezing and rhinorrhea, when exposed to cat or dust mites and have positive wheal-and-flare cutaneous tests to these same antigens. But IgE-mediated disease is absent in 40–70% of AERD patients and even in those patients with coexisting IgE-mediated respiratory disease; the identified allergens may not be available to initiate or propagate the chronic inflammation of AERD. This situation is most obvious in cat-sensitive AERD patients who do not own or visit a cat or have small children who bring home cat allergen on their clothing. These patients experience allergic symptoms after rare exposure to cat allergen. However, the chronic eosinophilic inflammatory disease of AERD could not be the result of rare cat exposure. One could argue that dust mite sensitivity is a different situation, since exposure to dust mites is ubiquitous in most localities. But even in these AERD patients, their inflammatory mucosal disease persists at high altitudes, where dust mites cannot survive.

Could this be a genetic disease? Are there genetic differences in the regulation of enzyme systems in inflammatory cells that are exclusively found in AERD patients? Or, as suggested by Prof. Szczeklik (61), is AERD the result of a chronic or latent viral infection of the respiratory tract, with activation of T-lymphocytes, secretion of cytokines (IL-3, IL-5 and IL-13), followed by recruitment, activation, and infiltration of eosinophils and mast cells? Indeed, patients with AERD almost universally describe a typical viral “cold” at the onset of their respiratory disease, which usually occurs between the ages of 20–40 yr of age (60). By the time patients are evaluated by physicians who are interested in this research, electron microscopy studies have not demonstrated viral particles in the mucosa of AERD patients. However, such a lack of evidence does not eliminate the possibility that the disease began as a common viral “cold,” an event that is indistinguishable from the millions of persons who have 1–2

colds every winter. However, unlike normal individuals, AERD patients may lack a fundamental ability to turn off the inflammatory response to the inciting virus. There is a report of a herpes simplex infection immediately preceding the onset of AERD (62). However, this virus is not usually associated with onset of respiratory disease in most patients with AERD, and proving that the HV was concomitant rather than causative is not possible.

A reasonable but unproven hypothesis is that the onset of the respiratory disease of AERD is the result of a combination of environmental or infectious events plus genetic abnormalities. Both components could work together, with genetic defects rendering the individual susceptible to or unable to recover from an otherwise routine viral respiratory illness or environmental exposure.

The Genetic Control of LTC₄ Synthase Enzymes

Figure 2 presents the arachidonic acid cascade. Any enzyme in the cascade, beginning with the phospholipases and ending with terminal enzymes, is a potential candidate for a genetically controlled increase or decrease in eicosanoid synthesis. Intuitively, patients with AERD may be expected to have an increase in activity of one or more essential enzymes that would drive the arachidonic cascade toward overproduction of leukotrienes. If such a genetic or developmental abnormality were limited to AERD patients, a logical explanation for overproduction of lipid mediators or prostanoids downstream from an upregulated enzyme might then be proposed. Furthermore, such a finding could be a genetically programmed difference that distinguishes AERD patients from normals and ASA-tolerant asthmatics. Thus, in AERD patients, the critical enzymes, which could enhance overproduction of leukotrienes include phospholipase A₂, 5-LO, 5-LO, activating protein, and leukotriene C₄ synthase (LTC₄S).

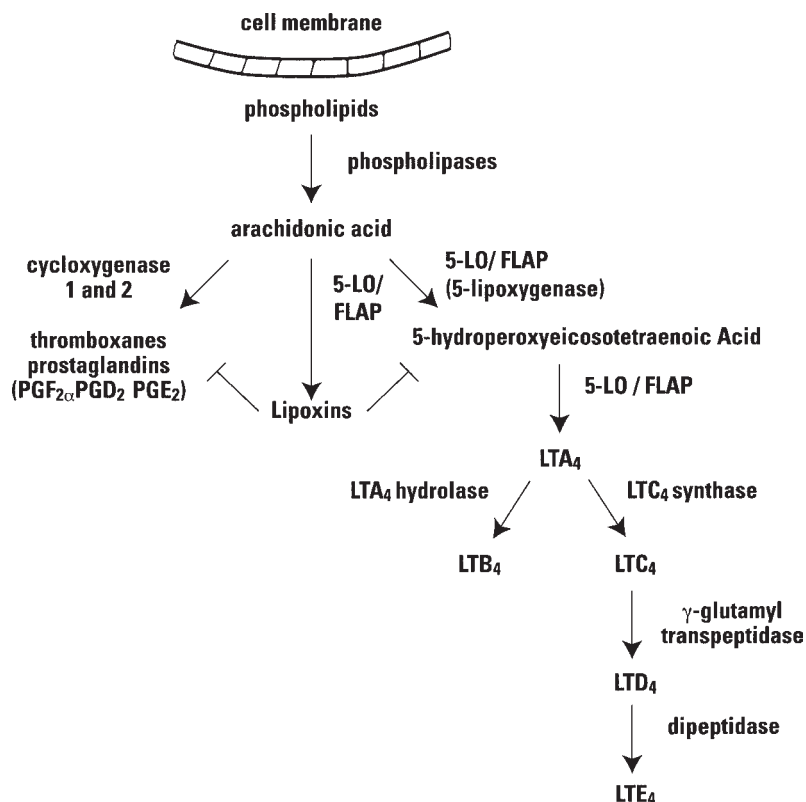


Fig. 2. Arachidonic acid products are synthesized into either the COX or 5-LO pathways. LTB₄ is preferentially formed in PMNs, as are lipoxins. Lipoxins are inhibitory, and turn off the arachidonic acid cascade. Mast cells and eosinophils are the main source of LTC₄ synthase, the critical enzyme in directing synthesis of LTC₄, D₄, E₄. COX-1 is found in most mammalian cells, including epithelial and endothelial cells in the respiratory tract and provides a steady source of PGE₂. COX-2 is restricted to inflammatory cells and cytokines, and other products of infection upregulate its synthesis.

Genetic Polymorphism

The human genome is continuously reshaped by heritable mutations, which are passed onto the next generation. If more than one allele is present at a given chromosome locus, and they differ by a single basepair, such a mutation is referred to as a single-nucleotide polymorphism (SNP). Other sequence variants—such as repeats, insertions and deletions—also occur, but SNPs are responsible for the majority of phenotypic differences. SNPs are most suitable for association studies (which can determine phenotypic effects of complex genetic abnormalities), rather than linkage analysis, because they are frequent, stable, and amenable to high-throughput genotyping. Many SNPs are common, occurring in the human

genome with a frequency of 10%, and most variants are functionally silent (63). The average distribution of SNPs is about one SNP for every 1000 basepairs, and the number of SNPs estimated in the human genome is between 50,000 and 100,000. More than 1.5 million SNPs have been found in the human genome, and the number increases exponentially as genetic sequencing advances (63).

LTC₄ Synthase

In 1997, Sanak and his colleagues (64) reported that 60% of AERD patients from Poland were found to have a SNP in the 5' regulatory region of the LTC₄ synthase gene. This polymorphism consisted of an adenine (A) to cytosine (C) transversion at -444 nucleotides upstream to the ATG translation start

site in the promoter region of the LTC₄ synthase gene. The -444C allele was associated with increased transcription and over-expression of LTC₄ enzymes in the affected AERD patients (65). Although no relationship was seen between the LTC₄ genotype and secretion of urinary LTE₄ in AERD patients at baseline, inhalation challenge with ASA-lysine induced a striking and preferential increase in urinary LTE₄ in AERD patients who were carriers of the -444C allele (66). Immunostaining of bronchial biopsies from AERD asthmatics, ASA-tolerant asthmatics and normal individuals for relevant inflammatory enzymes revealed no differences in the three study populations for: 5-LO, its activator protein (FLAP), COX-1, or COX-2. However, counts of cells, which stained for LTC₄ synthase, were 5× higher in the AERD asthmatics with the -444C allele (34). Therefore, these findings offered a possible explanation as to why AERD patients synthesized excessive quantities of leukotrienes (67). It is important to note, however, that 40% of the Polish AERD study subjects did not carry the -444C allele, and that 25% of a normal control population in Poland also carried the -444C allele, with no evidence of health problems, including asthma or ASA-induced respiratory reactions (64). Additionally, in a collaborative study, using DNA samples from patients in the United States and Japan, Van Sambeek et al. (68) were unable to confirm an increased prevalence of the -444C allele in these populations of AERD patients, when compared to appropriate control groups. Therefore, the -444C allele in the LTC₄S gene did not appear to correlate with the phenotypic expression of aspirin-sensitive asthma or the transcriptional activity of the LTC₄S gene.

In a recent study by Kawagishi et al. (69), polymorphism of the LTC₄ gene was evaluated in Japanese patients with aspirin-induced asthma (AIA) and aspirin-tolerant asthma (ATA). A variety of important data were presented. First, the genotype frequency of the -444C allele of the LTC₄S gene was 0.192 for

AERD patients, 0.110 for aspirin-tolerant asthma (ATA), and 0.200 for normal controls. Statistically, there were no difference between normals and AERD patients, but ATA patients had a significantly decreased frequency compared to both groups. Of the 60 AERD patients, 20 (33%) were either homozygous or heterozygous for the -444C allele. The population of AERD patients who carried the -444C allele was then compared to the population of AERD patients who were homozygous for -444A. Clinically, the -444C group was distinguished because they were younger, with a longer duration of asthma than the 40 patients with the -444A genotype. However, there were no differences in sex, predicted FEV₁, or use of inhaled or systemic corticosteroids. As expected, the mean urine LTE₄ levels were higher in the AERD group than in the ATA or normals, but within the AERD groups there was tremendous spread and no difference in mean values for LTE₄. Interestingly, although there was a lower prevalence of the -444C allele in ATA patients, they produced significantly more LTE₄ in the urine than the normals with the same frequency of the -444C allele as the AERD group. There was also no difference in the LTC₄S activity in the eosinophils from AERD patients and normal controls, but both were increased over eosinophil LTC₄S activity from ATA patients. Thus, polymorphisms for LTC₄ synthase are not responsible for overproduction of leukotrienes in any AERD patients, or else the variant LTC₄ synthase gene may be one of several abnormalities, with other abnormal alleles to be discovered in other populations of AERD patients (70).

Kawagishi et al. (69) also studied mutations in the 5-LO gene in the same subjects, and again were unable to find any differences between the three groups. To date, there has been no convincing evidence for upregulation of any of the critical enzymes in the arachidonate cascade in AERD subjects, except LTC₄ synthase. This statement does not imply pessimism in the pursuit of SN polymorphisms in AERD patients. In

fact, we believe it is likely that other genetic abnormalities are present in AERD patients, and that over time they will be discovered.

This discussion leads to a curious conclusion about ASA respiratory disease. Either the disease is the result of a single abnormal gene or an altered regulatory system, or it is a collection of genetic and environmental defects that have the same clinical presentation. The evidence for the first theory does not exist, which does not assure that future discoveries might provide the proper support for ASA disease as a single pathologic entity. However, at the present time, it is not possible to combine all of the abnormalities, cited in this section into a single pathogenic sequence for all AERD patients. The probability remains that various pathways can converge into stimulation of mast cells and eosinophils, with multiple defects or upregulations leading to the final inflammatory disorder.

Pathogenesis of ASA-Induced Respiratory Reactions

Clinical Presentations of Reactions and Their Pathologic Implications

Respiratory reactions to ASA and NSAIDs can best be divided into lower respiratory, upper respiratory, and systemic. Although ASA-induced reactions in upper-respiratory or lower-respiratory tracts can occur separately, a substantial majority of AERD patients experience reactions in both upper and lower respiratory tracts ("classic reactions") (71). During oral challenges with ASA in AERD patients, systemic reactions do not appear by themselves, but are added onto the predominant respiratory reactions. This is in contrast to patients who have anaphylaxis to ASA or an NSAID, for whom the systemic reaction is predominant and respiratory responses are a side issue. Nevertheless, by dissecting the respiratory reactions and the associated systemic reactions into specific organ responses, we gain some useful insight into the pathogenic events that lead to the respiratory reactions.

Lower Respiratory Reactions to ASA

These reactions consist of bronchospasm, with the intensity of the reactions strongly related to the dose of ASA or NSAIDs. The degree of bronchospasm, particularly after ingesting full therapeutic doses of ASA (650 mg) can be profound and even life-threatening. ASA-induced bronchospasm typically lasts for several hours, and is only partially responsive to beta agonist treatment. The clinical presentation of bronchospasm during an ASA-induced reaction is identical to that observed during inhalation of leukotrienes (52,72). In contrast to histamine inhalation, LTE_4 by inhalation induces a dose response, which was 13 times greater than histamine (52). In general, ASA-induced bronchospasm is more severe than histamine-induced bronchospasm, lasts for hours, and responds poorly to bronchodilators. Thus, inhalation of leukotrienes reproduces a clinical picture that is identical to the dose response, ASA-induced bronchospasm. Finally, if the same provoking dose of ASA is used at baseline and after pretreatment with either a cysLT_1 -receptor antagonist or a 5-LO-blocking drug, the second ASA-induced reaction is completely blocked (53,73,74). By contrast, pretreatment with a potent antihistamine had no protective effect on ASA-induced bronchospasm (75).

Upper Respiratory Reactions to ASA

These reactions consist of two parts. The first component is severe nasal congestion with paranasal headaches. The second component is sneezing, profuse rhinorrhea, and ocular injection with pruritis of the conjunctivae. Both histamine and leukotrienes are released into nasal secretions during ASA-induced upper-respiratory reactions (76–79). Pretreatment with an antihistamine eliminated most of the upper airway reactions, although nasal congestion was not specifically measured (75). Pretreatment with leukotriene-modifying drugs did not effect upper-airway reactions in a large series of AERD patients undergoing oral ASA chal-

lenges (80). The ASA-induced upper-airway reactions appear to be mediated by both histamine (the predominant mediator) and leukotrienes.

Systemic Reactions to ASA During Predominant Respiratory Reactions

During inhalation challenges with ASA-lysine, reproducible reactions in the lower airway (81–84) or upper airway (79,85) can be produced. During inhalation challenges with ASA, it is extraordinarily rare to have any systemic symptoms. In fact, if the lower airway is challenged with ASA-lysine, the upper airway does not react, and the opposite is true if the upper airway is challenged with ASA-lysine.

By contrast, systemic reactions are relatively common during oral challenges with ASA. At the Scripps Clinic, during the last 200 oral ASA challenges, 48/200 (24%) of the patients experienced some type of extra-respiratory reactions in addition to their respiratory reactions. Most of these reactions were relatively minor, with flushing 25/200 (12.5%) or gastrointestinal (cramping abdominal pain, nausea, or diarrhea) in 25/200 (12.5%) accounting for most of these responses. Eleven (5%) had transient hives that cleared completely as they reached ASA desensitization, and 7 (3%) had generalized pruritis. Flushing and hypotension, in addition to a respiratory reaction, occurred in one patient. Most systemic reactions involved more than one organ, although 21 patients were single-organ reactors, usually with flushing or gastrointestinal (GI) symptoms. These extra respiratory reactions are almost certainly the result of the release of histamine from the respiratory tract into the systemic circulation. Bosso et al. (86) measured serum tryptase and plasma histamine levels in 17 ASA-sensitive rhino-sinusitis asthmatic patients during oral ASA challenges. Three of 17 patients experienced both nasocular and bronchospastic reactions and also had systemic reactions (flushing, diarrhea, and cramping abdominal pain). Plasma and serum from only these three patients contained sig-

nificant elevations of plasma histamine and serum tryptase levels. Notably, these three patients experienced the same magnitude of respiratory reactions when compared to the remaining 14 patients. Sladek et al. (30) also measured increased serum tryptase during ASA-provoked bronchospastic reactions. Szczeklik et al. (75) were able to show that pretreatment with clemastine, an older first-generation antihistamine, prevented flushing reactions during oral ASA-induced respiratory reactions. In our series of 200 patients, who experienced systemic reactions during ASA-induced respiratory reactions, one-half had already been pretreated with leukotriene-receptor antagonists. Thus, LTRAs did not protect against systemic reactions. Our clinical experience in treating and preventing these systemic reactions has been highly successful, using intravenous (iv) H_1 blockers such as diphenhydramine, and H_2 blockers such as ranitidine. All of these observations seem to implicate histamine as the major—and perhaps the only—mediator responsible for systemic reactions in AERD patients.

Initiation of Reactions to ASA

Based on crossreactivity of AERD patients to structurally distinct NSAIDs upon first exposure, as well as the failure of selective COX-2 inhibitors to induce reactions (87,88), inhibition of COX-1 appears to be the initial signal that initiates an ASA-induced respiratory reaction (6) (see Fig. 1). Therefore, considerable attention has been focused on possible differences between the expression or function of COX enzymes in AERD asthmatics and tolerant asthmatic controls. In vitro studies of peripheral monocytes from AERD patients, taken during ASA-induced reactions, showed that inhibition of COX-1 could be demonstrated after in vivo exposure to as little as 60 mg of ASA given orally (89). Much larger doses—in the range of 325–650 mg given orally—were required to inhibit COX in monocytes from ASA-tolerant asthmatics and normal volunteers. This observation could not

have been the result of a more efficient absorption and distribution of ASA in AERD patients because serum levels of salicylates post-ASA challenge were found to be the same in normals and AERD patients (90,91). Therefore, the possibility that cyclooxygenase (COX) enzymes in AERD patients may be different (more vulnerable to acetylation by ASA or inhibition by NSAIDs) is an attractive differentiating theory to explain why AERD patients are highly susceptible to these drugs. Regardless of whether or not this theory is true, removing COX-1 (acetylation by ASA or channel blockade by NSAIDs) appears to initiate the ASA/NSAID-induced respiratory reaction.

As shown in Fig. 1, a hypothesis that might explain ASA/NSAID-induced respiratory reactions, is as follows: Overproduction of leukotrienes as a result of the inflammatory disease of AERD is already underway for reasons that are not clearly elucidated. Very small concentrations of ASA/NSAIDs rapidly inhibit COX-1 in AERD patients, abrogating additional synthesis of COX products, including PGE₂. Under the influence of phospholipases A₂ (PLA₂), arachidonic acid from cell membranes is constantly available. Since PGE₂ is known to have potent inhibitory effects on 5-LO, inhibition of PGE₂ synthesis, in the face of continued generation of archidonate, leads to increased synthesis of leukotrienes (6,61). The evidence for this sequence of events is very persuasive. In BAL fluid obtained from AERD asthmatics, the instillation of ASA-lysine into the bronchial segment is followed by a sharp decline in PGE₂ (36). Nasal secretions from AERD patients had measurable levels of PGE₂ at baseline. However, after oral ASA challenges, PGE₂ levels fell rapidly to undetectable levels (76). Urine thromboxane B₂ disappeared after ASA inhalation challenge challenges in AERD subjects, suggesting a rapid inhibition of COX-1 by ASA in the respiratory tract (28). Sestini et al. (92) showed that ASA-lysine-provoked bronchospasm was associated with a rise in urine LTE₄. However, after pretreat-

ment with inhaled PGE₂, ASA-lysine by inhalation did not induce bronchospasm, and there was no expected increase in urine LTE₄. In the same year, similar experiments by Szczeklik et al. (93) showed that both PGE₂ and salmeterol pretreatment prevented ASA-lysine-induced bronchospasm. Pretreatment with oral misoprostol (synthetic PGE₂) prevented ASA-induced asthma during oral challenges with ASA (94).

The important and unanswered question is: do AERD patients have a peculiar susceptibility to depletion of COX-1 by ASA and NSAIDs or do they start with a defective synthetic system, which is synthesizing less PGE₂ at baseline? Picado et al. (45) discovered that mRNA for COX-2 was significantly reduced in nasal polyp cells from AERD patients, when compared to healthy normal mucosa and nasal polyps from aspirin-tolerant asthmatics. The authors suggested that AERD patients were vulnerable to further depletion of PGE₂ by ASA because of the low baseline production of COX-2-dependent prostanoids. Kowalski demonstrated that cultured epithelial cells from AERD patients generated less PGE₂ than cells from controls (95). However, a recent study by Antczak et al. (96) presents data that is substantially different from these studies. By measuring mediators in the exhaled breath condensate, they were able to demonstrate that PGE₂ concentrations were the same in AERD asthmatics and ASA-tolerant asthmatics. Their conclusion was that a deficiency of PGE₂ was unlikely to be an important mechanism in the pathogenesis of ASA-induced reactions.

There is also substantial evidence that mast cells contribute to acute ASA-induced reactions by discharging tryptase and histamine (76,77,86). Ferreri et al. (76) measured increased LTC₄ and histamine and decreased PGE₂ in nasal lavage fluid after oral ASA challenges in AERD asthmatics. Both histamine and LTC₄ appeared in the nasal secretions slightly before the onset of naso-ocular reactions. Similar findings of increased histamine and LTC₄ during oral challenges with ASA were reported by

Kowalski et al. (78) and after intranasal challenges with ASA-lysine by Picado et al. (79). Fischer et al. (77) induced nasal reactions by oral ASA challenges, and measured significantly increased concentrations of tryptase, histamine, and LTC₄ in the nasal secretions during the reactions. However, ECP was not increased when compared to controls. Although the evidence that mast cells participate in ASA-induced nasal reactions is impressive, the mechanism by which ASA induces histamine and tryptase release from mast cells is not clear. One explanation is that activated mast cells are stabilized by PGE₂, and when synthesis of this prostanoids is interrupted, mast cells are no longer inhibited, and discharge their granule mediators (6). This explanation begs the question as to why AERD mast cells are so unstable in the first place? Could the explanation be that ASA directly acetylates mast-cell membranes, which then provides a signal to initiate granule discharge?

Similar findings have been reported in studies of the lower respiratory tract in AERD patients. Nasser et al. (97), demonstrated that endobronchial challenge with ASA-lysine decreased the numbers of non-degranulated mast cells and increased the number of activated eosinophils compared to baseline numbers. Sladek et al. (12) obtained BAL fluid 30 min after ASA-lysine inhalation challenges in 10 AERD asthmatics. Following challenge, BAL fluid contained increased concentrations of eosinophil cationic protein, LTC₄, and histamine, and decreased concentrations of TXB₂.

In 1991, Christie et al. (26) studied AERD patients after oral ASA challenges, and found significant increases in urinary LTE₄, peaking at 6 h post-ASA-induced respiratory reactions. These observations have been confirmed, using both oral and inhalation ASA challenges (28,30,98). Daffern et al. (31) showed that baseline levels of urinary LTE₄ predicted the severity of the subsequent ASA-induced lower-respiratory reactions. High baseline levels of LTE₄ were associated with severe asthmatic reactions, in

which the FEV₁ declined >30%, whereas low baseline levels were followed by ASA-induced naso-ocular reactions only. Sladek et al. (30) demonstrated a rapid decline in urinary TXB₂ and an increase in LTE₄, during oral ASA-induced bronchospastic reactions. Studies by Szczeklik and Sestini (92,93), as presented earlier in this section, showed that added PGE₂, by inhalation prevented ASA-induced respiratory reactions. Therefore, reduction of PGE₂ synthesis by inhibition of COX-1 appears to be an important initiating event in LT overproduction and the subsequent respiratory reactions.

Epithelial Cells from Nasal Polyps of Patients with AERD

Cultured epithelial cells obtained from nasal polyps were examined for arachidonic acid metabolism before and after calcium ionophore stimulation. Unstimulated epithelial cells from AERD patients generated similar quantities of PGF_{2α} and 15-HETE as compared to ASA-tolerant epithelial cells. However, AERD epithelial cells generated significantly less PGE₂, both spontaneously and after stimulation with calcium ionophore. After stimulation with ionophore, cell cultures from both groups increased synthesis of PGE₂, 15-HETE, and PGF_{2α}, without synthesis of PGD₂ or LTC₄. After adding ASA, only the epithelial cells from AERD patients generated increased 15-HETE. Simultaneously, the addition of ASA resulted in a decrease in PGE₂ in cells from both AERD patients and tolerant controls (99). The finding that ASA stimulates 15-HETE generation in AERD patients appears to differentiate AERD patients from control asthmatics, but the mechanism by which diversion of arachidonate into 15-HETE might induce respiratory reactions is unknown. However, the quantitative loss of protection, because of the lower levels of PGE₂, increased the vulnerability of AERD patients to a greater depletion of PGE₂ by ASA.

Susceptibility to the Effects of Leukotrienes

Arm et al. (52) evaluated bronchial airway responsiveness to histamine and LTE_4 in AERD asthmatics and found a 13-fold increase in airway responsiveness to inhaled LTE_4 , when compared to ASA-tolerant asthmatics. There were no differences between the two groups in their responses to inhaled histamine. Therefore, airways of AERD asthmatics appeared to be preferentially sensitive to the effects of this terminal LT, further magnifying the degree of bronchospasm that occurs during ASA-induced reactions. Thus during ASA/NSAID-induced respiratory reactions, new molecules of leukotrienes are generated, and cysLT_1 receptors on bronchial smooth muscle of AERD patients appear to be able to augment leukotriene signals. A potential explanation for this phenomenon is that AERD patients have different cysLT_1 receptors, which can more efficiently bind and/or signal a smooth-muscle response. No published literature exists to support this theory.

In the nasal tissues, similar challenge studies with LTE_4 before and after ASA challenges have not been conducted. However, the upper airway may not yield clear information from LTE_4 -challenge studies because of the considerable role of histamine and the confounding presence of a second and potentially a third cysLT receptor.

Mechanisms to Explain Aspirin Desensitization

Acute ASA Desensitization

Mechanisms accounting for ASA desensitization are only partly understood. A few facts exist, but most questions remain unanswered. During acute ASA desensitization, defined as 3 h after first ingestion of ASA 650 mg with no adverse effects, peripheral-blood monocytes synthesized significantly less thromboxane B_2 but only slightly less LTB_4 , the preferential 5-LO product of peripheral monocytes (100). At

acute ASA desensitization, COX-1 and perhaps COX-2 are completely inactivated, with very little if any PGE_2 available to inhibit leukotriene synthesis. This is supported by the following study. In a large series of AERD asthmatics, levels of LTE_4 in the urine were the same at acute ASA desensitization as they were at baseline (31). However, on the first day following ASA desensitization, Arm et al. (52) demonstrated a 20-fold decrease in bronchial airway responsiveness to LTE_4 , compared to pre-desensitization responses in AERD asthmatics. These two experiments suggested that ASA desensitization downregulates cysLT_1 receptors in the bronchial smooth muscles; so that although leukotrienes are available, they can no longer induce a strong signal. In a study of ASA induced release of histamine and tryptase, Bosso et al. (86) showed that these mediators disappeared at acute desensitization, suggesting that mast cell discharge was completed or ASA in high doses directly or indirectly interfered with discharge of granule mediators. The ability to tolerate ASA and other NSAIDs at acute ASA desensitization remains somewhat of a mystery. The phenomenon cannot be explained by further depletion of PGE_2 synthesis or interruption of leukotriene synthesis. At the same time, significant decongestion of the nasal tissues at acute desensitization strongly suggests that ASA is directly inhibiting mast cells from releasing histamine and PGD_2 and at the same time ASA is blocking cysLT_1 and perhaps cysLT_2 receptors in the endothelial cells of the nasal vessels or mucus-secreting glands. Further studies are needed to unravel this complex series of interactions between ASA and the inflammatory, vascular, and secretory mechanisms that have been described here.

Chronic ASA Desensitization

Chronic desensitization occurs after 2 wk of treatment by daily ingestion of ASA in AERD patients who have completed ASA desensitization. As shown in a recent clinical study (101), improvement in clinical symptoms and sense

of smell occurred within the first month of ASA desensitization treatment. Jurgens et al. (100) showed that after treatment with ASA 650 mg BID for 2 wk or more, LTB₄ synthesis in peripheral monocytes declined substantially, to the same level as found in normal controls. Nasser, et al. (102) reported that during chronic ASA desensitization, urine LTE₄ levels declined to lower values, but not to levels found in normal controls. Thus, leukotrienes in these ASA-treated patients were still present in sufficient quantities to stimulate cysLT receptors.

With these limited facts, what are the possible ways in which ASA treatment could account for improved symptoms, particularly decreased nasal congestion and improved sense of smell, in AERD patients who are treated with daily ASA for many months? It seems to us that we should be looking at three main areas that might yield useful information on this subject. The first is that ASA might acetylate or downregulate cysLT₁ and ₂ receptors on endothelial cells, mucus glands, and eosinophils, decreasing nasal congestion and mucus secretion and gradually turning down the eosinophilic cycle of infiltration and recruitment. Certainly, systemic corticosteroids effectively decrease eosinophilic infiltration into respiratory tissues in AERD patients, and although the mechanisms may not be the same, the end result—a comparison of ASA and systemic corticosteroid treatment—could be similar.

The second possibility is that higher ASA doses of 650 mg BID are enough to inhibit the upregulated inflammatory COX-2 enzyme in inflammatory cells. This presumes a role for COX-2 products in the genesis of the inflammatory disease of AERD in the first place. In support of this theory is the fact that only 81 mg per d of ASA will maintain the desensitized state, a dose capable of inhibiting COX-1 but not COX-2. By contrast, therapeutic doses of ASA, in the range of 650–1300 mg/d of ASA are required to reduce nasal congestion, a dose compatible with inhibition of COX-2. The third possibility is that ASA has other direct effects

on inflammatory cells, which are independent of those effects described here. For example, ASA has been reported to inhibit activation of the transcription factor NF-kappa B (103) and also to inhibit IL-4 and IL-13 induced activation of STAT6 (104). Future studies will be important because the scarcity of data about the effects of ASA desensitization currently inhibit progress in understanding these important mechanisms.

References

1. Vane, J. R. (1971), Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nat. New Biol.* **231**, 232–235.
2. Zembowicz, A., Jones, S. L., and Wu, K. K. (1995), Induction of cyclooxygenase-2 in human umbilical vein endothelial cells by lysophosphatidylcholine. *J. Clin. Investig.* **96**, 1688–1692.
3. Hawley, C. (1999), COX-2 inhibitors. *Lancet* **353**, 307–314.
4. Samuelsson, B., Hammarstroem, S., Murphy, R. C., and Borgeat, P. (1980), Leukotrienes and slow reacting substance of anaphylaxis (SRS-A). *Allergy* **35**, 375–381.
5. Samuelsson, B., Dahlen, S. E., Lindgren, J. A., Rouzer, C. A., and Serhan, C. N. (1987), Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. [Review.] *Science* **237**, 1171–1176.
6. Szczeklik, A. and Stevenson, D. D. (1999), Aspirin-induced asthma: advances in pathogenesis and management. *J. Allergy Clin. Immunol.* **104**, 5–13.
7. Moneret-Vautrin, D. A., Hsieh, V., Wayoff, M., Guyot, J. L., Mouton, C., and Maria, Y. (1990), Nonallergic rhinitis with eosinophilia syndrome a precursor of the triad: nasal polyposis, intrinsic asthma, and intolerance to aspirin. *Ann. Allergy* **64**, 513–518.
8. Yamashita, T., Tsuyi, H., Maeda, N., Tomoda, K., and Kumazawa, T. (1989), Etiology of nasal polyps associated with aspirin-sensitive asthma. *Rhinology* **8**, 15–24.
9. Nasser S. M., Pfister, R., Christie, P. E., Sousa, A. R., Barker, J., Schmitz-Schumann, M., et al. (1996), Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Am. J. Respir. Crit. Care Med.* **153**, 90–96.
10. Sousa, A., Pfister, R., Christie, P. E., Lane, S. J., Nasser, S. M., Schmitz-Schumann, M., et al. (1997), Enhanced expression of cyclo-oxygenase isoenzyme 2 (COX-2) in asthmatic airways and its cellular distribution in aspirin-sensitive asthma. *Thorax* **52**, 940–945.
11. Sousa, A. R., Lams, B. E., Pfister, R., Christie, P. E., Schmitz, M., and Lee, T. H. (1997), Expression

- of interleukin-5 and granulocyte-macrophage colony-stimulating factor in aspirin-sensitive and non-aspirin-sensitive asthmatic airways. *Am. J. Respir. Crit. Care Med.* **156**, 1384–1389.
12. Sladek, K., Dworski, R., Soja, J., Sheller, J. R., Nizankowska, E., Oates, J. A., et al. (1994), Eicosanoids in bronchoalveolar lavage fluid of aspirin-intolerant patients with asthma after aspirin challenge. *Am. J. Respir. Crit. Care Med.* **149**, 940–946.
 13. Kowalski, M., Grzegorzczak, J., Wojciechowska, B., and Sponiatowska, M. (1996), Intranasal challenge with aspirin induces cell influx and activation of eosinophils and mast cells in nasal secretions of ASA-sensitive patients. *Clin. Exp. Allergy* **26**, 807–814.
 14. Borish, L. (2002), Sinusitis and asthma: entering the realm of evidence-based medicine. *J. Allergy Clin. Immunol.* **109**, 606–607.
 15. Hamilos, D., Leung, D. Y. M., Wood, R., Cunningham, L., Bean, D. K., and Yasruel, Z. (1995), Evidence for distinct cytokine expression in allergic versus non-allergic chronic sinusitis. *J. Allergy Clin. Immunol.* **96**, 537–544.
 16. Bachert, C., Wagenmann, M., Hauser, U., and Rudack, C. (1997), IL-5 synthesis is upregulated in human nasal polyp tissue. *J. Allergy Clin. Immunol.* **99**, 837–842.
 17. Kowalski, M. L., Grzegorzczak, J., Pawliczak, R., Kornatowski, T., Wagrowska-Danilewicz, M., and Danilewicz, M. (2002), Decreased apoptosis and distinct profile of infiltrating cells in the nasal polyps of patients with aspirin hypersensitivity. *Allergy* **57**, 493–500.
 18. Bachert, C., Wagenmann, M., Rudack, C., Hopken, K., Hillebrandt, M., Wang, D., and Van Cauwenberge, P. (1998), The role of cytokines in infectious sinusitis and nasal polyposis. *Allergy* **53**, 2–13.
 19. Settipane, G. A. (1987), Nasal polyps: pathology, immunology and treatment. *Am. J. Rhinol.* **1**, 119–126.
 20. Stevenson, D. and Simon, R. A. Sensitivity to aspirin and nonsteroidal antiinflammatory drugs, in *Allergy: Principles and Practice*, Middleton, E., Jr., Ellis, E. F., Yunginger, J. W., Reed, C. E., Adkinson, N. F., Jr., and Busse, W. W., eds., vol 2, 5th ed., Mosby, St. Louis, 1998, pp. 1225–1234.
 21. Stevenson, D. (2000), Approach to the patient with a history of adverse reactions to aspirin or NSAIDs: diagnosis and treatment. *Allergy and Asthma Proceedings* **21**, 25–31.
 22. Kakoi, H. and Hiraide, F. A. (1987), A histologic study of formation and growth of nasal polyps. *Acta Otolaryngol.* **103**, 137–144.
 23. Bachert, C., Gevaert, P., and Van Cauwenberge, P. (1999), Nasal polyposis—a new concept on the formation of polyps. *ACI International* **11**, 130–135.
 24. Elsner, J., Hochstetter, R., Kimming, D., and Kapp, A. (1996), Human eotaxin represents a potent activator of the respiratory burst in human eosinophils. *Eur. J. Immunol.* **26**, 1919–1925.
 25. Mullol, J., Fernandez-Morata, J. C., Roca-Ferrer, J., Pujols, L., Xaubet, A., Benitez, P., et al. (2002), Cyclooxygenase 1 and 2 expression is abnormally regulated in human nasal polyps. *J. Allergy Clin. Immunol.* **109**, 824–830.
 26. Christie, P. E., Tagari, P., Ford-Hutchinson, A. W., Charlesson, S., Chee, P., Arm, J. P., et al. (1991), Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. *Am. Rev. Respir. Dis.* **143**, 1025–1029.
 27. Smith, C. M., Hawksworth, R. J., Thien, F. C., Christie, P. E., and Lee, T. H. (1992), Urinary leukotriene E4 in bronchial asthma. *Eur. Respir. J.* **5**, 693–699.
 28. Kumlin, M., Dahlen, B., Bjorck, T., Zetterstrom, O., Granstrom, E., and Dahlen, S. E. (1992), Urinary excretion of leukotriene E4 and 11-dehydro-thromboxane B2 in response to bronchial provocations with allergen, aspirin, leukotriene D4, and histamine in asthmatics. *Am. Rev. Respir. Dis.* **146**, 96–103.
 29. Knapp, H. R., Sladek, K., and Fitzgerald, G. A. (1992), Increased excretion of leukotriene E4 during aspirin-induced asthma. *J. Lab. Clin. Med.* **119**, 48–51.
 30. Sladek, K. and Szczeklik, A. (1993), Cysteinyl leukotrienes overproduction and mast cell activation in aspirin-provoked bronchospasm in asthma. *Eur. Respir. J.* **6**, 391–399.
 31. Daffern, P., Muilenburg, D., Hugli, T. E., and Stevenson, D. D. (1999), Association of urinary leukotriene E4 excretion during aspirin challenges with severity of respiratory responses. *J. Allergy Clin. Immunol.* **104**, 559–564.
 32. Kohno, S. (1998), Lipid mediators in bronchial asthma. *Allergy Clin. Immunol. International* **10**, 181–186.
 33. Schmid, M., Gode, U., Schafer, D., and Wigand, M. E. (1999), Arachidonic acid metabolism in nasal tissue and peripheral blood cells in aspirin intolerant asthmatics. *Acta Otolaryngol.* **119**, 277–280.
 34. Cowburn, A. S., Sladek, K., Soja, J., Adamek, L., Nizankowska, E., Szczeklik, A., et al. (1998), Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J. Clin. Invest.* **101**, 834–846.
 35. Takasaki, J. K. M. and Matsumoto, M. (2000), The molecular characterization and tissue distribution of the human cysteinyl leukotriene cys LT (2) receptor. *Biochem. Biophys. Res. Commun.* **274**, 316–322.
 36. Szczeklik, A., Sladek, K., Dworski, R., Nizankowska, E., Soja, J., and Oates, J. (1996), Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am. J. Respir. Crit. Care Med.* **154**, 1608–1614.
 37. Braccioni, F., Dorman, S. C., O'Byrne, P. M., Inman, M. D., Denburg, J. A., Parameswaran, K., et al.

- (2002), The effect of cysteinyl leukotrienes on the growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects. *J. Allergy Clin. Immunol.* **110**, 96–101.
38. Spada, C., Nieves, A. L., Krauss, A. H., and Woodward, D. F. (1994), Comparison of leukotriene B₄ and D₄ effects on human eosinophil and neutrophil motility in vitro. *J. Leukoc. Biol.* **55**, 183–191.
39. Mita, H., Endoh, S., Kudoh, M., Kawagishi, Y., Kobayashi, M., Taniguchi, M., et al. (2001), Possible involvement of mast-cell activation in aspirin provocation of aspirin-induced asthma. *Allergy* **56**, 1061–1067.
40. Ferreri, N. R., Howland, W. C., and Spiegelberg, H. L. (1986), Release of leukotrienes C₄ and B₄ and PGE₂ from human monocytes stimulated with aggregated IgG, IgA and IgE. *J. Immunol.* **136**, 4188–4193.
41. Ziroli, N. E., Na, H., Chow, J. M., Stankiewicz, J. A., Samter, M., and Young, M. R. (2002), Aspirin-sensitive versus non-aspirin-sensitive nasal polyp patients: analysis of leukotrienes/Fas and Fas ligand expression. *Otolaryngol. Head Neck Surg.* **126**, 141–146.
42. Holtzman, M. J., Turk, J., and Shornick, L. P. (1992), Identification of a pharmacologically distinct prostaglandin H synthase in cultured epithelial cells. *J. Biol. Chem.* **267**, 21,438–21,445.
43. Demoly, P., Jaffuel, D., Lequex, B., Cremion, C., Michel, F., Godard, P., et al. (1997), Prostaglandin H synthase 1 and 2 immunoreactivities in the bronchial mucosa of asthmatics. *Am. J. Respir. Crit. Care Med.* **155**, 670–675.
44. Smith, W. L. and Dewitt, D. L. (1996), Cyclooxygenase enzymes: a review. *Adv. Immunol.* **62**, 167–215.
45. Picado, C., Fernandez-Morata, J. C., Juan, M., Roca-Ferrer, J., Fuentes, M., Xaubet, A., et al. (1999), Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am. J. Respir. Crit. Care Med.* **160**, 291–296.
46. Lynch, K. R., O'Neill, G. P., Liu, Q., Im, D. S., Sawyer, N., Metters, K. M., et al. (1999), Characterization of the human cysteinyl leukotriene CysLT₁ receptor. *Nature* **399**, 789–793.
47. Saru, H., Ames, R. S., Chambers, J., Ellis, C., Elshourbagy, N., and Foley, J. J. (1999), Identification, molecular cloning, expression, and characterization of a cysteinyl leukotriene receptor. *Mol. Pharmacol.* **56**, 657–663.
48. Figueroa, D. J., Breyer R. M., Defoe, S. K., Kargman, S., Daugherty, B. L., Waldburger, K., Liu, Q., et al. (2001), Expression of the cysteinyl leukotriene 1 receptor in normal human lung and peripheral leukocytes. *Am. J. Respir. Crit. Care Med.* **163**, 226–233.
49. Heise, C. E. O. D. B. and Figuero, D. J. (2000), Characterization of the human cysteinyl leukotriene 2 receptor. *J. Biol. Chem.* **275**, 30,531–30,536.
50. James, A. and Sampson, A. P. (2001), A tale of Two CysLTs. *Clin. Exp. Allergy* **31**, 1660–1664.
51. Mita, H., Hasegawa, M., Saito, H., and Akiyama, K. (2001), Levels of cysteinyl leukotriene receptor mRNA in human peripheral leukocytes: significantly higher expression of cysteinyl leukotriene receptor 2 mRNA in eosinophils. *Clin. Exp. Allergy* **31**, 1716–1725.
52. Arm, J. P., O'Hickey, S. P., Spur, B. W., and Lee, T. H. (1989), Airway responsiveness to histamine and leukotriene E₄ in subjects with aspirin-induced asthma. *Am. Rev. Respir. Dis.* **140**, 148–153.
53. Christie, P. E., Smith, C. M., and Lee, T. H. (1991), The potent and selective sulfidopeptide leukotriene antagonist, SK&F 104353, inhibits aspirin-induced asthma. *Am. Rev. Respir. Dis.* **144**, 957–958.
54. Stevenson, D., Simon, R. A., Mathison, D. A., and Christiansen, S. C. (2000), Montelukast is only partially effective in inhibiting aspirin responses in aspirin sensitive asthmatics. *Ann. Allergy Asthma Immunol.* **85**, 477–482.
55. Dahlen, S., Malstrom, K., Nizankowska, E., Dahlen, B., Kuna, P., Kowalski, M., et al. (2002), Improvement of aspirin-intolerant asthma by montelukast, a leukotriene antagonist. A randomised, double blind, placebo controlled trial. *Am. J. Respir. Crit. Care Med.* **165**, 9–14.
56. Szczeklik, A., Nizankowska, E., Mastalerz, L., and Bochenek, G. (2002), Myocardial ischemia possibly mediated by cysteinyl leukotrienes. *J. Allergy Clin. Immunol.* **109**, 572–573.
57. Serhan, C. N. (2001), Lipoxins and aspirin-triggered 15-epi-lipoxins are endogenous components of anti-inflammation: emergence of the counterregulatory side. *Arch. Immunol. Ther. Exp.* **49**, 177–188.
58. Sanak, M., Levy, B. D., Clish, C. B., Chiang, N., Gronert, K., Mastalerz, L., et al. (2000), Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics. *Eur. Respir. J.* **16**, 44–49.
59. Szczeklik, A., Nizankowska, E., and Duplaga, M. (2000), Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. *Eur. Respir. J.* **16**, 432–436.
60. Berges-Gimeno, M., Simon, R. A., and Stevenson, D. D. (2002), The natural history and clinical characteristics of aspirin exacerbated respiratory disease. *Ann. Allergy Asthma Immunol.* **89**, 474–478.
61. Szczeklik, A. (1990), The cyclooxygenase theory of aspirin-induced asthma. [Review.] *Eur. Respir. J.* **3**, 588–593.
62. Nakagawa, H., Yoshida, S., Nakabayashi, M., Akahori, K., Shoji, T., Hasegawa, H., et al. (2001), Possible relevance of virus infection for development of analgesic idiosyncrasy. *Respiration* **68**, 422–424.
63. Brookes, A. (1999), The essence of SNPs. *Gene* **234**, 177–186.
64. Sanak, M., Simon, Hans-Uwe, and Szczeklik, A. (1997), Leukotriene C₄ synthase promotor polymorphism and risk of aspirin-induced asthma. *Lancet* **350**, 1599–1600.

65. Penrose, J. F. and Baldasaro, M. H. (1999), Leukotriene C4 synthase: a candidate gene for the aspirin-intolerant asthmatic phenotype. *Allergy Asthma Proc.* **20**, 353–360.
66. Szczeklik, A., Sanak, M., Nizankowska, E., Mastalerz, L., Bochenek, G., and Pulka, G. (1998), Leukotriene C4 synthase genetic polymorphism directs urinary cysteinyl-leukotiene response to aspirin challenge in asthma. *Allergy* **53**, 61–67.
67. Sanak, M. and Szczeklik, A. (2000), Genetics of aspirin induced asthma. *Thorax* **55**, S45–S47.
68. Van Sambeek, R. S. D., Baldasaro, M., Lam, B. K. Z. J., Yoshida, S., Yandora, C., and Drazen, J. M. P. J. (2000), 5' flanking region polymorphism of the gene encoding leukotriene C4 synthase does not correlate with the aspirin-intolerant asthma phenotype in the United States. *J. Allergy Clin. Immunol.* **106**, 72–76.
69. Kawagishi, K., M. H., Taniguchi, M., Maruyama, M., Oosaki, R., Higashi, N., Kashii, T., et al. (2002), Leukotriene C4 synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. *J. Allergy Clin. Immunol.* **109**, 936–942.
70. Bigby, T. (2000), The leukotriene C4 synthase gene and asthma. *Am. J. Respir. Cell Mol. Biol.* **23**, 273–276.
71. Pleskow, W. W., Stevenson, D. D., Mathison, D. A., Simon, R. A., Schatz, M., and Zieger, R. S. (1983), Aspirin-sensitive rhinosinusitis/asthma: spectrum of adverse reactions to aspirin. *J. Allergy Clin. Immunol.* **71**, 574–579.
72. Lewis, R. A. and Austen, K. F. (1984), The biologically active leukotrienes: biosynthesis, metabolism, receptors, functions, and pharmacology. *J. Clin. Investig.* **73**, 889–902.
73. Dahlen, B. J., Kumlin, M., Margolskee, D., Larsson, C., Blomqvist, H., Williams, V. C., et al. (1993), The leukotriene receptor antagonist MK-0679 blocks airway obstruction induced by bronchial provocation with lysine-aspirin in aspirin-sensitive asthmatics. *Eur. Respir. J.* **6**, 1018–1026.
74. Israel, E., Fischer, A. R., Rosenberg, M. A., Lilly, C. M., Callery, J. C., Shapiro, J., et al. (1993), The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. *Am. Rev. Resp. Dis.* **148**, 1447–1451.
75. Szczeklik, A. and Serwonska, M. (1979), Inhibition of idiosyncratic reactions to aspirin in asthmatic patients by clemastine. *Thorax* **34**, 654–658.
76. Ferreri, N. R., Howland, W. C., Stevenson, D. D., and Spiegelberg, H. L. (1988), Release of leukotrienes, prostaglandins, and histamine into nasal secretions of aspirin-sensitive asthmatics during reaction to aspirin. *Am. Rev. Respir. Dis.* **137**, 847–854.
77. Fischer, A. R., Rosenberg, M. A., Lilly, C. M., Callery, J. C., Rubin, P., Cohn, J., et al. (1994), Direct evidence for a role of the mast cell in the nasal response to aspirin in aspirin-sensitive asthma. *J. Allergy Clin. Immunol.* **94**, 1046–1056.
78. Kowalski, M. L., Sliwiska-Kowalska, M., Igarashi, Y., White, M. V., Wojciechowska, B., Brayton, P., et al. (1993), Nasal secretions in response to acetylsalicylic acid. *J. Allergy Clin. Immunol.* **91**, 580–598.
79. Picado, C., Ramis, I., Rosello, J., Prat, J., Bulbena, O., Plaza, V., et al. (1992), Release of peptide leukotriene into nasal secretions after local instillation of aspirin in aspirin-sensitive asthmatic patients. *Am. Rev. Respir. Dis.* **145**, 65–69.
80. Berges-Gimeno, M., Simon, R. A., and Stevenson, D. D. (2002), The effect of leukotriene modifier drugs on asa-induced asthma and rhinitis reactions. *Clin. Exp. Allergy*.
81. Bianco, S. R. M. and Petrini, G. (1977), Aspirin induced tolerance in aspirin-asthma detected by a new challenge test. *IRCS J. Med. Sci.* **5**, 129–136.
82. Schmitz-Schumann, V. M., Juhl, E., and Costabel, U. (1985), Analgesic asthma-provocation challenge with acetylsalicylic acid. *Atemwegs-Lungenkrankh Jahrgang* **10**, 479–485.
83. Kuna, P., Zielinska, E., Bpchska-Marciniak, M., and Rozniecki, J. (1997), Early and late asthmatic response after inhalation challenge with lysine-ASA in subjects with aspirin-induced asthma. *J. Allergy Clin. Immunol.* **99**, S411 (Abstract #1669).
84. Dahlen, B. and Melillo, G. (1998), Inhalation challenge in ASA-induced asthma. *Respir. Med.* **92**, 378–384.
85. Patriarca, G., Nucera, E., and Di Rienzo, V. (1991), Nasal provocation test with lysine acetylsalicylate (LAS) in aspirin-sensitive patients. *Ann. Allergy* **67**, 60–62.
86. Bosso, J. V., Schwartz, L. B., and Stevenson, D. D. (1991), Tryptase and histamine release during aspirin-induced respiratory reactions. *J. Allergy Clin. Immunol.* **88**, 830–837.
87. Stevenson, D. and Simon, R. A. (2001), Lack of cross-reactivity between rofecoxib and aspirin in aspirin sensitive asthmatic patients. *J. Allergy Clin. Immunol.* **108**, 47–51.
88. Woessner, K., Simon, R. A., and Stevenson, D. D. (2002), The safety of celecoxib in aspirin exacerbated respiratory disease. *Arthritis Rheum.* **46**, 2201–2206.
89. Juergens, U. R., Christiansen, S. C., Stevenson, D. D., and Zuraw, B. L. (1992), Arachidonic acid metabolism in monocytes of aspirin-sensitive asthmatic patients before and after oral aspirin challenge. *J. Allergy Clin. Immunol.* **90**, 636–645.
90. Stevenson, D. D., Arroyave, C. M., Bhat, K. N., and Tan, E. M. (1976), Oral aspirin challenges in asthmatic patients: a study of plasma histamine. *Clin. Allergy* **6**, 493–505.
91. Dahlen, B., Boreus, L. O., Anderson, P., Andersson, R., and Zetterstrom, O. (1994), Plasma acetylsalicylic acid and salicylic acid levels during aspirin provocation in aspirin-sensitive subjects. *Allergy* **49**, 43–49.
92. Sestini, P., Armetti, L., Gambaro, G., Pieroni, M. G., Refini, R. M., Sala, A., et al. (1996), Inhaled PGE2

- prevents aspirin-induced bronchoconstriction and urinary LTE₄ excretion in aspirin-sensitive asthma. *Am. J. Respir. Crit. Care Med.* **153**, 572–575.
93. Szczeklik, A., Mastalerz, L., Nizankowska, E., and Cmiel, A. (1996), Protective and bronchodilator effects of prostaglandin E and salbutamol in aspirin-induced asthma. *Am. J. Respir. Crit. Care Med.* **153**, 567–571.
94. Szmidt, M. and Wasiak, W. (1996), The influence of misoprostol (synthetic analogue of prostaglandin E₁) on aspirin-induced bronchoconstriction in aspirin-sensitive asthma. *J. Investig. Allergol. Clin. Immunol.* **6**, 121–125.
95. Kowalski, M., Ptasiński, A., Bienkiewicz, B., and DuBuske, L. M. (2002), Aspirin-induced 15-HETE generation is a specific aspirin-sensitive asthma/rhinosinusitis patient identification test. *J. Allergy Clin. Immunol.* **209**, S30.
96. Antczak, A., Montuschi, P., Kharitonov, S., Gorski, P., and Barnes, P. J. (2002), Increased exhaled cysteinyl-leukotrienes and 8-isoprostane in aspirin-induced asthma. *Am. J. Respir. Crit. Care Med.* **166**, 301–306.
97. Nasser, S., Christie, P. E., Pfister, R., Sousa, A. R., Walls, A., Schmitz-Schumann, M., et al. (1996), Effect of endobronchial aspirin challenge on inflammatory cells in bronchial biopsy samples from aspirin-sensitive asthmatic subjects. *Thorax* **51**, 64–70.
98. Christie, P. E., Tagari, P., Ford-Hutchinson, A. W., Black, C., Markendorf, A., Schmitz-Schumann, M., et al. (1992), Urinary leukotriene E₄ after lysine-aspirin inhalation in asthmatic subjects. *Am. Rev. Respir. Dis.* **146**, 1531–1534.
99. Kowalski, M. L., Pawliczak, R., Wozniak, J., Siuda, K., Poniatowska, M., Iwaszkiewicz, J., et al. (2000), Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am. J. Respir. Crit. Care Med.* **161**, 391–398.
100. Juergens, U. R., Christiansen, S. C., Stevenson, D. D., and Zuraw, B. L. (1995), Inhibition of monocyte leukotriene B₄ production following aspirin desensitization. *J. Allergy Clin. Immunol.* **96**, 148–156.
101. Berges-Gimeno, M., Simon, R. A., and Stevenson, D. D. (2003), Early effects of aspirin desensitization treatment in asthmatics with aspirin exacerbated respiratory disease. *Ann. Allergy Clin. Immunol.*
102. Nasser, S. M. S., Patel, M., Bell, G. S., Lee, T. H. (1995), The effect of aspirin desensitization on urinary leukotriene E₄ concentration in aspirin-sensitive asthma. *Am. J. Respir. Crit. Care Med.* **151**, 1326–1330.
103. Kopp, E. and Ghosh, S. (1994), Inhibition of NF- κ B by sodium salicylate and aspirin. *Science* **265**, 956–959.
104. Perez, G. M., Melo, M., Keegan, A. D., and Zamorano, J. (2002), Aspirin and salicylates inhibit the IL-4 and IL-13 induced activation of STAT6. *J. Immunol.* **168**, 1428–1434.