

Pathogenesis of nonsteroidal antiinflammatory drug-induced asthma

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Purpose of review

To summarize recent findings related to the pathogenic mechanisms of aspirin-induced asthma with emphasis on molecular genetic mechanisms.

Recent findings

The overproduction of cysteinyl leukotrienes with the increased expression of cysteinyl leukotriene receptor 1 (CYSLTR1) is a consistent finding in aspirin-induced asthma patients. Recent data have suggested a dysregulation of cyclooxygenase-2 and prostaglandin E₂, increased levels of 15-hydroxyeicosatetraenoic acid, and decreased lipoxin generation as characteristics of the condition. The HLA allele DPB1*0301 was documented as a strong genetic marker for susceptibility in an Asian population. Leukotriene C4 synthase has been established as a key genetic determinant of aspirin-induced asthma, but recent studies have demonstrated that several single nucleotide polymorphisms in the promoters of prostaglandin E₂ receptor subtype 2, CYSLTR1 and CYSLTR2 and T-box expressed in T cells (TBX21) could increase risk for the condition. Although cyclooxygenase-2 and thromboxane A2 receptor polymorphisms were not associated with aspirin-induced asthma phenotype, they may exert functional effects.

Summary

The identification of genetic markers for aspirin-induced asthma susceptibility along with in-vitro functional studies would help to elucidate the pathogenesis of the condition. Further studies of the interactions among genes and between genes and the environment will be essential.

Keywords

aspirin-induced asthma, gene polymorphism, leukotriene

Abbreviations

15-HETE	15-hydroxyeicosatetraenoic acid
AIA	aspirin-induced asthma
ALOX5	5-lipoxygenase
ALOX5AP	5-lipoxygenase activating protein
ASA	acetyl salicylic acid
ATA	aspirin-tolerant asthma
COX-2	cyclooxygenase 2
Cys-LT	cysteinyl leukotriene
EP2	prostaglandin E ₂ receptor subtype 2
FEV1	forced expiratory volume in 1 s
LTC4S	leukotriene C4 synthase
NSAID	nonsteroidal antiinflammatory drug
SNP	single nucleotide polymorphism
TBX21	T-box expressed in T cells
TBXA2R	thromboxane A2 receptor

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Introduction

Aspirin (acetyl salicylic acid; ASA) and other nonsteroidal antiinflammatory agents (NSAIDs) cause bronchoconstriction in 10–20% of adults with asthma [1,2]. This clinical syndrome has been termed ASA-induced asthma (AIA). AIA is commonly found in nonatopic, middle-aged females and is characterized by a typical constellation of symptoms, including moderate to severe asthma, intense eosinophilic inflammation of nasal and bronchial tissues, and higher prevalence of chronic rhinosinusitis and nasal polyposis [3]. The diagnosis of AIA can be established with certainty by challenge tests with increasing doses of ASA. There are four types of challenge tests, depending on the route of ASA administration: oral, bronchial, nasal and, rarely, intravenous tests. Both the oral and the bronchial tests have similar specificity, but the oral test has somewhat higher sensitivity [4]. To avoid the possibility of a severe reaction to an oral ASA challenge, the lysine-ASA bronchoprovocation test is widely used in European and Asian countries. As an in-vitro test, the measurement of ASA-induced 15-hydroxyeicosatetraenoic acid (15-HETE) in peripheral blood was suggested as a specific test for the identification of ASA-sensitive patients (ASPI test) [5*]. Most major pathogenic studies of AIA have focused on altered eicosanoid metabolisms. After the European network on AIA reported the occurrence of familial ASA hypersensitivity in 5.1% of the patients in their large survey [6], the number of molecular genetic studies conducted to identify genetic markers for the susceptibility to AIA has increased. Despite these efforts, the pathogenicity of AIA is still not fully understood.

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Cellular and biochemical mechanisms of aspirin-induced asthma

Persistent airway inflammation with intense eosinophil infiltration and the evidence of Cys-LT overproduction are characteristic findings of AIA, and many studies have been attempting to explore cellular and biochemical mechanisms.

Overproduction of cysteinyl leukotrienes and increased expression of cysteinyl leukotriene receptor 1

ASA/NSAIDs block the cyclooxygenase pathway, causing arachidonate substrates to be diverted to the 5-lipoxygenase (ALOX5, or 5-LO) pathway. In this pathway, arachidonate, liberated from membrane phospholipids by cell activation, is converted to the leukotriene LTA₄ by ALOX5 and its cofactor 5-lipoxygenase activating protein (ALOX5AP or FLAP). The products of this pathway can induce chemotaxis of inflammatory cells via LTB₄ and cysteinyl leukotrienes (Cys-LTs, LTC₄/D₄/E₄). The underlying profound actions of these Cys-LTs include bronchoconstriction, vascular permeability, elevated mucus secretion, eosinophil recruitment, smooth muscle hypertrophy, and fibrous collagen depositions [7,8]. LTC₄ synthase (LTC₄S) is the terminal enzyme in the production of Cys-LT and is markedly overexpressed in eosinophils and mast cells of the airway mucosa of AIA patients [9]. Bronchial biopsies from AIA patients revealed a fourfold increase in eosinophils in comparison with the level in ASA-tolerant asthma (ATA) patients, and the baseline concentrations of Cys-LTs measured in the bronchoalveolar lavage (BAL) fluid of AIA patients correlated well with the counts of LTC₄S-positive cells in the bronchial mucosa [9,10]. AIA patients were more sensitive to inhaled Cys-LTs. Cys-LTs are also released into the nasal cavity after nasal challenge with ASA [11] and into bronchi after challenge with lysine-ASA [12]. Most patients with AIA excrete between two and tenfold higher amounts of LTE₄ in their urine than do patients with ATA [13]. Therefore, Cys-LTs have emerged as major mediators in AIA pathogenesis.

The biological activities of Cys-LTs are exerted as consequences of binding to their receptors, CYSLTR1 and CYSLTR2, on surfaces of inflammatory cells [14,15]. Recent novel studies have demonstrated that the number of cells expressing CYSLTR1 in the nasal mucosa was significantly higher in AIA patients than in ASA-tolerant patients, suggesting that an enhanced response of inflammatory cells to Cys-LTs due to the overexpression of CYSLTR1 may contribute to the pathogenesis of ASA hypersensitivity [16]. They also suggested the effects of Cys-LTs on glands and epithelium may be mediated through CYSLTR2 due to differential distribution between CYSLTR1 and CYSLTR2 in the nasal mucosa [17•]. Thus, a clue to the underlying anomaly in ASA intolerance can be provided by studying the overproduc-

tion of Cys-LTs and the increased expression of CYSLTR1 and CYSLTR2.

Dysregulation of cyclooxygenase and prostaglandins

The cyclooxygenase enzyme, which is central to the mechanism of aspirin intolerance, exists in at least two isoforms, COX-1 and COX-2, which are encoded by distinct genes. ASA and most NSAIDs inhibit both enzymes, but are much more potent inhibitors of COX-1 than COX-2. Specific COX-2 inhibitors can be tolerated by AIA patients. The overall expression of COX-1 and COX-2 was similar in bronchial biopsies obtained from AIA and ATA patients [18–21]. The two enzymes were coexpressed in the same cell types, that is, bronchial epithelia, smooth muscle cells and fibroblasts; however, their proportions could differ greatly [22]. One major difference identified in cyclooxygenase expression was the downregulation of COX-2 expression in the nasal polyp tissue of AIA patients, suggesting COX-2 dysregulation in AIA [23].

The inhalation of the prostaglandin PGE₂ could inhibit ASA-induced bronchoconstriction and reduced the LTE₄ level in the urine of AIA patients [24]. The basal PGE₂ production by nasal epithelial cells was lower in AIA patients, and this difference was obvious after exposure to ASA, suggesting that the nasal polyp epithelium from AIA patients demonstrates an intrinsic defect in its capacity to generate PGE₂ [25]. A lower production of PGE₂ was also noted in the peripheral blood cells [26,27] and cultured bronchial fibroblasts of ASA-intolerant rhinitis patients [28]. Moreover, lower PGE₂ production in airway smooth muscle cells was accompanied by the simultaneous downregulation of COX-2 mRNA expression [29]. These findings suggest that NSAIDs may enhance the depletion of this protective prostaglandin, PGE₂, in association with the downregulation of COX-2.

Inflammatory cells are more activated in aspirin-induced asthma

Eosinophil infiltration of upper and lower airway tissue is a central feature of AIA. An elevated serum interleukin (IL)-5 level [30] and an enhanced local production of IL-5 and granulocyte-macrophage colony-stimulating factor were shown in the bronchial mucosa of AIA patients [30,31]. Increased numbers of mast cells as well as eosinophils were found in the bronchial mucosa of AIA patients [32,33]. The PGD₂ concentration in AIA patients was significantly higher than in ATA patients [34]. When a segmental bronchial challenge with ASA was performed in AIA and ATA patients [12], ASA significantly depressed PGE₂ and thromboxane B₂ levels in the BAL fluids of both groups; however, the levels of PGD₂, PGF₂α and 9α, 11α-PGF₂ decreased only in ATA patients. Modest increases were seen in the urinary levels

of 9α , 11α -PGF₂ and PGD metabolites in AIA patients, suggesting the involvement of activated mast cells as well as eosinophils.

The 15-lipoxygenase pathway:

15-hydroxyeicosatetraenoic acid and lipoxin

It was reported that the production of 15-HETE by peripheral blood leukocytes in AIA patients is 3.6-fold higher than in ATA patients and that 15-HETE production is enhanced in AIA patients after ASA exposure [35]. Among the potent proinflammatory activities of 15-HETE are the stimulation of proinflammatory mediators from mast cells [36], the induction of mucous glycoprotein secretion by human airway [37], and the contraction of human bronchial smooth muscle [18]. 15-HETE, however, also has antiinflammatory activities, including the inhibition of 5-LO activity, the generation of LTB₄ in leukocytes [19,20], and the production of lipoxin which inhibits eosinophil chemotaxis [21]. The mechanisms by which ASA induces 15-HETE production and the consequent clinical course are not understood.

Lipoxins, antiinflammatory derivatives of arachidonic acid, are derived from the 15-LO pathway and behave as functional antagonists of leukotrienes [38]. Lipoxin production was decreased in AIA patients compared with that in ATA patients [39]. The increased action of Cys-LTs in patients with AIA might be attributable to a diminished capacity for lipoxin synthesis [38,39], a possible factor contributing to the clinical course of AIA [21].

Genetic mechanism of aspirin-induced asthma

To explore the genetic determinants of AIA, association studies of candidate genes, of leukotriene-related genes, in particular, have been undertaken. Most studies have been focused on promoter polymorphisms and nonsynonymous single nucleotide polymorphisms (SNPs).

Leukotriene C4 synthase gene

Two promoter polymorphisms in the LTC₄S gene (−1072G>A, −444A>C) were identified, of which LTC₄S−444A>C was associated with the AIA phenotype in a Polish population [40,41]. The homozygous LTC₄S−444A allele was associated with a greater increase in urinary LTE₄ after an ASA challenge in a Japanese population [42], and this genetic variation had an additional responsive element for histone H4 transcription factor-2, which increased the in-vivo and in-vitro transcription rate of LTC₄S. This has not been replicated in other populations, however, including US, Japanese, and Korean populations [42,43,44*].

The 5-lipoxygenase gene and leukotriene-related genes

In *et al.* [45] identified a 5-LO (ALOX5) gene promoter polymorphism, which consisted of a variable number of

tandem repeats of GC-rich motifs in association with binding sites for Sp1 transcription factor; individuals with the wild genotype containing five repeats had a significantly higher capacity to produce Cys-LTs than did those with the mutant genotypes containing three, four, or six repeats. This polymorphism was found to be associated with the severity of airway hyperresponsiveness in a Korean population [46]; AIA patients carrying a mutant allele containing less than or greater than five repeats showed increased airway hyperresponsiveness compared with those with the wild genotype. In addition, AIA patients were screened for 10 SNPs in key enzymes involved in arachidonate metabolism: 5-lipoxygenase (ALOX5; −1708G>A, 21C>T, 270G>A, 1728G>A), 5-lipoxygenase activating protein (ALOX5AP; 218A>G), cyclooxygenase 2 (COX-2; −162C>G, 10T>G, 228G>A), leukotriene C4 synthase (LTC4S; −444A>C), and cysteinyl leukotriene receptor 1 (CYSLTR1; 927T>C). Based on the Korean population, a possible involvement of ALOX5-ht1 [G–C–G–A] in AIA patients was suggested; the frequency of the [G–C–G–A] haplotype of ALOX5 was significantly higher in AIA patients than in ATA. A lack of association was noted between ALOX5AP, COX-2, and CYSLTR1 gene polymorphisms and AIA phenotype [44*].

Cysteinyl leukotriene receptor genes

Cys-LTs exert their biological actions by binding to two types of receptors – CYSLTR1 and CYSLTR2. Three SNPs in the 5' upstream region of the CYSLTR1 gene (−634C>T, −475A>C, −336A>G) were identified [47]. Male AIA patients had higher frequencies of the minor alleles of these three SNPs than did males in the control group, while no differences were noted in the female patients. A functional study using two cell lines, human T-lymphocyte cell (jurkat) and lung epithelial cell (A549), demonstrated enhanced promoter activity with the ht2 [T–C–G] construct compared with that of the ht1 [C–A–A] construct, suggesting that this polymorphism could modulate CYSLTR1 expression to increase AIA susceptibility. Four SNPs were identified in the CYSLTR2 gene, c.−819T>G, c.2078C>T, c.2534A>G, and c.2545+297A>G, from a Korean population [48*]. In that study, the frequencies of rare alleles for c.−819T>G, c.2078C>T, and c.2534A>G were higher in AIA patients than in ATA patients. The patients with rare alleles for c.−819T>G, c.2078C>T or c.2534A>G exhibited a more pronounced fall in forced expiratory volume in 1 s (FEV1) after ASA provocation.

Cyclooxygenase-2 pathway: cyclooxygenase, prostaglandin E2 and thromboxan A2 receptor gene

A case–control study of 63 candidate genes was conducted in a Japanese population [49**]. A functional SNP of PGE₂ receptor subtype 2 (EP2) gene was associated with AIA by reducing the braking effect of PGE₂

on inflammation. A novel promoter polymorphism (−765G>C) of COX-2 has been recently identified in a Polish population [50^{*}]. The promoter polymorphism of COX-2 showed a significant association with sex, atopy, and an increased PGE₂ production in AIA patients.

The thromboxane A₂ receptor (TBXA₂R) is a receptor for a potent bronchoconstrictor, TBXA₂. The TBXA₂R +795T>C polymorphism may increase bronchoconstrictive response to ASA, which could contribute to the development of the AIA phenotype in the Korean population [51^{*}]. Increased production of Cys-LTs in AIA patients can be explained by a mechanism via inhibition of ongoing formation of platelet-derived TBXA₂ [52]. That study indicated that oral aspirin administration can lead to the uncoupling of the TBXA₂-dependent negative feedback mechanism, which may increase the production of Cys-LTs. The TBXA₂-dependent regulation of LTC₄S activity may be an important pathophysiological mechanism in AIA.

T-box expressed in T cells gene

The gene TBX21 encodes the transcription factor T-bet, which is T-box expressed in T cells. It influences naive T-lymphocyte development and has been implicated in asthma pathogenesis [53]. The −1993T T>C polymorphism in the TBX21 promoter was associated with an increased risk for AIA in a Japanese population by increasing transcriptional activity [54^{**}]. This genetic variation can cause inappropriate Th1 cell responses in the airway, leading to severe airway inflammation, suggesting that the Th1 cell response may play a role as great as that of the Th2 cell response in the pathogenesis of AIA. It was cautiously proposed that AIA may be classified roughly as Th1/Th2 mixed-type asthma.

HLA allele

The association of HLA DPB1*0301 with AIA has been reported in a Polish population [55]. The increased frequency of DPB1*0301 in AIA patients compared with that in normal controls and in controls with asthma suggests that immune recognition of an unknown antigen may be part of the pathogenesis of AIA. These findings were replicated in a Korean population [56^{**}]. The patients with DPB1*0301 tended to be female, have lower FEV₁, and show higher prevalence of rhinosinuitis or nasal polyp in comparison with patients without the allele. Furthermore, the presence of HLA DPB1*0301 was significantly associated with the requirement for leukotriene receptor antagonists in the long-term management of AIA patients [57^{*}].

Interaction between the candidate genes

Recent investigations have suggested gene–gene interaction or epigenetic regulation in the pathogenic mechanism of AIA. The genetic effects of CYSLTR2

c.2078C>T (or CYSLTR2 c.2534A>G) and LTC₄S–444A>C synergistically increased the decrease in FEV₁ after lysine-ASA inhalation, which strongly suggests that interactions between CYSLTR2 and LTC₄S may cooperatively influence the development of ASA intolerance in patients with asthma [48^{*}]. The TBXA₂R +795T>C polymorphism was significantly associated with the presence of HLA DPB1*0301 in AIA patients compared with ATA patients [51]. The TBXA₂R +924T>C polymorphism had no association with AIA, but the frequency of the mutant genotype combination of TBXA₂R +924T>C and ALOX5 +21C>T was significantly higher in AIA patients than in ATA patients or in normal controls. This result suggests that a gene–gene interaction of TBXA₂R and ALOX5 may be synergistically associated with the AIA phenotype. Further investigations are needed to determine how genes interact in the pathogenesis of AIA.

Conclusion

Further information on the genetic polymorphisms of candidate AIA-related genes along with supporting functional studies will help to elucidate the molecular mechanisms of AIA and may lead to the development of new diagnostic markers and additional therapeutic targets based on genetic information.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 74–76).

- 1 McDonald JR, Mathison DA, Stevenson DD. Aspirin intolerance, detection by oral challenge. *J Allergy Clin Immunol* 1972; 50:198–207.
- 2 Spector SL, Wangaard CH, Farr RS. Aspirin and concomitant idiosyncrasies in adult asthmatic patient. *J Allergy Clin Immunol* 1979; 64:500–506.
- 3 Park HS. Aspirin-sensitive asthma. *Bio Drugs* 2000; 13:29–33.
- 4 Szczeklik A, Nizankowska E, Sanak M, *et al.* Aspirin-induced rhinitis and asthma. *Curr Opin Allergy Clin Immunol* 2001; 1:27–33.
- 5 Kowalski ML, Ptasińska A, Jedrzejczak M, *et al.* Aspirin-triggered 15-HETE generation in peripheral blood leukocytes is a specific and sensitive aspirin-sensitive patients identification test (ASPISTest). *Allergy* 2005; 60:1139–1145.
- This study confirmed ASA-induced 15-HETE generation from peripheral blood leukocytes which can be used as an in-vitro test for aspirin-sensitive patients identification test (ASPISTest).
- 6 Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. AIA NE Investigators. European Network on Aspirin-Induced Asthma. *Eur Respir J* 2000; 16:432–436.
- 7 Henderson WR. The role of leukotrienes in inflammation. *Ann Intern Med* 1994; 121:684–697.
- 8 Lewis RA, Austen KF, Soberman RJ. Leukotrienes and other products of the 5-lipoxygenase pathway. *N Engl J Med* 1990; 323:645–655.
- 9 Cowburn AS, Sladek K, Soja J, *et al.* Overexpression of leukotriene C₄ synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J Clin Invest* 1998; 101:834–846.
- 10 Sampson AP, Cowburn AS, Sladek K, *et al.* Profound overexpression of leukotriene C₄ synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. *Int Arch Allergy Immunol* 1997; 113:355–357.
- 11 Ortolani C, Mirone C, Fontana A, *et al.* Study of mediators of anaphylaxis in nasal wash fluids after aspirin and sodium metabisulfite nasal provocation in intolerant rhinitic patients. *Ann Allergy* 1987; 59:106–112.

- 12 Szczeklik A, Sladek K, Dworski R, *et al.* Bronchial aspirin challenge causes specific eicosanoid response in aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1996; 154:1608–1614.
- 13 Smith CM, Hawksworth RJ, Thien FC, *et al.* Urinary leukotriene E4 in bronchial asthma. *Eur Respir J* 1992; 5:693–699.
- 14 Hui Y, Funk CD. Cysteinyl leukotriene receptors. *Biochem Pharmacol* 2002; 64:1549–1557.
- 15 Capra V. Molecular and functional aspects of human cysteinyl leukotriene receptors. *Pharmacol Res* 2004; 50:1–11.
- 16 Sousa AR, Parikh A, Scadding G, *et al.* Leukotriene receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. *N Engl J Med* 2002; 347:1493–1499.
- 17 Corrigan C, Mallett K, Ying S, *et al.* Expression of the cysteinyl leukotriene receptors cysLT(1) and cysLT(2) in aspirin-sensitive and aspirin-tolerant chronic rhinosinusitis. *J Allergy Clin Immunol* 2005; 115:316–322.
- This is the first study to localize CYSLTR2 in the nasal mucosa, which was different from that of CYSLTR1. They suggested that increased production of Cys-LTs might be mediated through CYSLTR2, especially in glands and epithelium.
- 18 Salari H, Schellenberg RR. Stimulation of human airway epithelial cells by platelet activating factor (PAF) and arachidonic acid produces 15-hydroxy-eicosatetraenoic acid (15-HETE) capable of contracting bronchial smooth muscle. *Pulm Pharmacol* 1991; 4:1–7.
- 19 Profita M, Sala A, Riccobono L, *et al.* 15(S)-HETE modulates LTB₄ (4) production and neutrophil chemotaxis in chronic bronchitis. *Am J Physiol Cell Mol Physiol* 2000; 279:C1249–C1258.
- 20 Schewe T, Petrich K, Ludwig P, *et al.* Effect of 15-HETE on the 5-lipoxygenase pathway in neutrophils: genuine inhibitor or alternative substrate? *Adv Exp Med Biol* 1999; 447:95–105.
- 21 Bandeira-Melo C, Bozza PT, Diaz BL, *et al.* Cutting edge: lipoxin (LX) A₄ and aspirin-triggered 15-epi-LXA₄ block allergen-induced eosinophil trafficking. *J Immunol* 2000; 164:2267–2271.
- 22 Taha R, Olivenstein R, Utsumi T, *et al.* Prostaglandin H synthase 2 expression in airway cells from patients with asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 161:636–640.
- 23 Picado C, Fernandez-Morata JC, Juan M, *et al.* Cyclooxygenase-2 mRNA is downexpressed in nasal polyps from aspirin-sensitive asthmatics. *Am J Respir Crit Care Med* 1999; 160:291–296.
- 24 Sestini P, Armetti L, Gambaro G, *et al.* Inhaled PGE₂ prevents aspirin-induced bronchoconstriction and urinary LTE₄ excretion in aspirin-sensitive asthma. *Am J Respir Crit Care Med* 1996; 153:572–575.
- 25 Kowalski ML, Pawliczak R, Wozniak J, *et al.* Differential metabolism of arachidonic acid in nasal polyp epithelial cells cultured from aspirin-sensitive and aspirin-tolerant patients. *Am J Respir Crit Care Med* 2000; 161:391–398.
- 26 Schmid M, Gode U, Schafer D, *et al.* Arachidonic acid metabolism in nasal tissue and peripheral blood cells in aspirin intolerant asthmatics. *Acta Otolaryngol* 1999; 119:277–280.
- 27 Schäfer D, Schmid M, Göde UC, *et al.* Dynamics of eicosanoids in peripheral blood cells during bronchial provocation in aspirin-intolerant asthmatics. *Eur Respir J* 1999; 13:638–646.
- 28 Pierzchalska M, Zsabo Z, Sanak M, *et al.* Deficient prostaglandin E₂ production by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma. *J Allergy Clin Immunol* 2003; 111:1041–1048.
- 29 Chambers LS, Black JL, Ge Q, *et al.* PAR-2 activation, PGE₂, and COX-2 in human asthmatic and nonasthmatic airway smooth muscle cells. *Am J Physiol Lung Cell Mol Physiol* 2003; 285:L619–L627.
- 30 Sousa AR, Lams BE, Pfister R, *et al.* 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* 1997; 156:1384–1389.
- 31 Varga EM, Jacobson MR, Masuyama K, *et al.* Inflammatory cell populations and cytokine mRNA expression in the nasal mucosa in aspirin-sensitive rhinitis. *Eur Respir J* 1999; 14:610–615.
- 32 Nasser SM, Pfister R, Christie PE, *et al.* Inflammatory cell populations in bronchial biopsies from aspirin-sensitive asthmatic subjects. *Am J Respir Crit Care Med* 1996; 153:90–96.
- 33 Nasser S, Christie PE, Pfister R, *et al.* Effect of endobronchial aspirin challenge on inflammatory cells in bronchial biopsy samples from aspirin-sensitive asthmatic subjects. *Thorax* 1996; 51:64–70.
- 34 Higashi N, Taniguchi M, Mita H, *et al.* A comparative study of eicosanoid concentrations in sputum and urine in patients with aspirin-intolerant asthma. *Clin Exp Allergy* 2002; 32:1484–1490.
- 35 Kowalski ML, Ptasińska A, Bienkiewicz B, *et al.* Differential effects of aspirin and misoprostol on 15-hydroxyeicosatetraenoic acid generation by leukocytes from aspirin-sensitive asthmatic patients. *J Allergy Clin Immunol* 2003; 112:505–512.
- 36 Goetzl EJ, Phillips MJ, Gold WM. Stimulus specificity of the generation of leukotrienes by dog mastocytoma cells. *J Exp Med* 1983; 158:731–737.
- 37 Marom Z, Shelhamer JH, Kaliner M. Effects of arachidonic acid, mono-hydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways in vitro. *J Clin Invest* 1981; 67:1695–1702.
- 38 Sanak M, Bazan-Socha S, Clish CB, *et al.* Altered lipoxin and 15-epi-lipoxin A₄ production by peripheral blood eosinophils in human asthmatics. *Am J Respir Crit Care Med* 1999; 159:A91.
- 39 Sanak M, Levy BD, Clish CB, *et al.* Aspirin-tolerant asthmatics generate more lipoxins than aspirin-intolerant asthmatics. *Eur Respir J* 2000; 16:44–49.
- 40 Sanak M, Simon HU, Szczeklik A. Leukotriene C₄ synthase promoter polymorphism and risk of aspirin-induced asthma. *Lancet* 1997; 350:1599–1600.
- 41 Sanak M, Pierzchalska M, Bazan-Socha S, *et al.* Enhanced expression of the leukotriene C₄ synthase due to overactive transcription of an allelic variant associated with aspirin-intolerant asthma. *Am J Respir Cell Mol Biol* 2000; 23:290–296.
- 42 Kawagishi Y, Mita H, Taniguchi M, *et al.* Leukotriene C₄ synthase promoter polymorphism in Japanese patients with aspirin-induced asthma. *J Allergy Clin Immunol* 2002; 109:936–942.
- 43 Van Sambeek R, Stevenson DD, Baldasaro M, *et al.* 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* 2000; 106:72–76.
- 44 Choi JH, Park HS, Oh HB, *et al.* Leukotriene-related gene polymorphisms in ASA-intolerant asthma: an association with a haplotype of 5-lipoxygenase. *Hum Genet* 2004; 114:337–344.
- This study demonstrated the frequency of the ALOX5 haplotype (ht1<G–C–G–A>) in AIA was significantly higher than in ATA, which might contribute to the development of AIA in a Korean population.
- 45 In KH, Asano K, Beier D, *et al.* Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *J Clin Invest* 1997; 99:1130–1137.
- 46 Kim SH, Bae JS, Suh CH, *et al.* Polymorphism of tandem repeat in promoter of 5-lipoxygenase in ASA-intolerant asthma: a positive association with airway hyperresponsiveness. *Allergy* 2005; 60:760–765.
- 47 Kim SH, Park HS, Choi JH, *et al.* A strong association of three genetic variants of CYSLTR1 with ASA-intolerant asthma phenotype. *J Allergy Clin Immunol* 2005; 115:S134.
- 48 Park JS, Chang HS, Park CS, *et al.* Association analysis of cysteinyl-leukotriene receptor 2 (CYSLTR2) polymorphisms with aspirin intolerance in asthmatics. *Pharmacogenet Genomics* 2005; 15:483–492.
- This is the first study to demonstrate CYSLTR2 genetic variants could contribute to increasing the susceptibility to AIA and also these genetic variants were significantly associated with bronchial responsiveness to inhaled lysine ASA.
- 49 Jinnai N, Sakagami T, Sekigawa T, *et al.* Polymorphisms in the prostaglandin E₂ receptor subtype 2 gene confer susceptibility to aspirin-intolerant asthma: a candidate gene approach. *Hum Mol Genet* 2004; 13:3203–3217.
- This study adopted an extensive candidate gene analysis for identifying the most susceptible SNPs for ASA-intolerant asthma in the Japanese population and a functional SNP of the EP2 gene was found to increase the risk.
- 50 Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of COX-2 gene polymorphism G-765C in bronchial asthma. *J Allergy Clin Immunol* 2004; 114:248–253.
- This study presents a functional effect of genetic variants (–765G>C) of COX-2, leading to enhanced prostaglandin production, which may be more significant in women for the contributing phenotype of ASA-intolerant asthma.
- 51 Kim SH, Choi JH, Park HS, *et al.* Association of thromboxane A₂ receptor gene polymorphism with the phenotype of acetyl salicylic acid-intolerant asthma. *Clin Exp Allergy* 2005; 35:585–590.
- This study demonstrated that the frequency of TXA₂R +795T>C) was higher in AIA than in ATA and this genetic variant (+795T>C) of thromboxane A₂ receptor could augment the bronchoconstrictive response to inhaled lysine ASA.
- 52 Tornhamre S, Ehnage A, Kolbeck KG, *et al.* Uncoupled regulation of leukotriene C₄ synthase in platelets from aspirin-intolerant asthmatics and healthy volunteers after aspirin treatment. *Clin Exp Allergy* 2002; 32:1566–1573.

- 53 Szabo SJ, Kim ST, Costa GL, *et al.* A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell* 2000; 100:655–669.
- 54 Akahoshi M, Obara K, Hirota T, *et al.* Functional promoter polymorphism in the
•• TBX21 gene associated with aspirin-induced asthma. *Hum Genet* 2005; 117:16–26.

They identified 24 SNPs of the TBX21 gene, a Th1-specific transcriptional factor and among them, –1993 T>C was significantly associated with higher risk of AIA with increased transcriptional activity of TBX21 gene, suggesting that AIA may present Th1/Th2 mixed-type asthma.
- 55 Dekker JW, Nizankowska E, Schmitz-Schumann M, *et al.* Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. *Clin Exp Allergy* 1997; 27:574–577.
- 56 Choi JH, Lee KW, Oh HB, *et al.* HLA association in aspirin-intolerant asthma:
•• DPB1*0301 as a strong marker in a Korean population. *J Allergy Clin Immunol* 2004; 113:562–564.

This is the confirmative study that HLA DPB1*0301 can be a strong genetic marker of AIA in a Korean population, which was also associated with leukotriene modifier requirement in the long-term management of ASA-intolerant asthma.
- 57 Park HS, Kim SH, Sampson AP, *et al.* The HLA-DPB1*0301 marker might
• predict the requirement for leukotriene receptor antagonist in patients with aspirin-intolerant asthma. *J Allergy Clin Immunol* 2004; 114:688–689.

This study demonstrated that the AIA patients with HLA-DPB1*0301 required a higher dose of leukotriene receptor antagonist to maintain asthma control state for 6 months, suggesting that HLA DPB1*0301 may be used for predicting the requirement for leukotriene receptor antagonist in AIA patients.