

EDITORIAL

Clinical & Experimental Allergy

Leukotriene C₄ synthase: the engine of aspirin intolerance?

This editorial discusses the findings of the paper in this issue by Hirata et al. [12]

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Aspirin-induced asthma and aspirin-exacerbated respiratory disease (AERD) are two of several synonyms for the 'aspirin triad' of asthma, nasal polyposis and sensitivity to aspirin described almost 90 years ago [1]. While the names cite aspirin alone, patients with AERD typically experience severe bronchoconstriction and/or rhinoconjunctival reactions to many non-steroidal anti-inflammatory drugs (NSAID), even those not encountered previously. The reactions reflect non-allergic hypersensitivity as many AERD patients are not atopic and the reactions to NSAIDs are not generally IgE-mediated. While AERD typically does not emerge fully until early middle age, a meta-analysis indicates that not only 21% of adult asthmatics but also 5% of asthmatic children are aspirin-intolerant [2]. Even when NSAIDs are entirely avoided, AERD patients have comparatively severe chronic asthma compared with aspirin-tolerant patients, with a high proportion requiring long-term oral corticosteroid therapy [1].

The cyclooxygenase (COX) theory of aspirin intolerance holds that the ability of NSAIDs to trigger adverse reactions in AERD patients correlates with their potency in inhibiting the COX-1 isozyme that generates prostanoids constitutively in many tissues, including the airway. Selective inhibitors of COX-2 appear not to trigger acute reactions [3]. As arachidonic acid, the substrate of the COX pathway, is also the initial substrate of the 5-lipoxygenase (5-LO) pathway that generates the leukotriene family of inflammatory mediators, NSAIDs are proposed to 'shunt' arachidonate away from prostanoid synthesis towards the synthesis of cysteinyl-leukotrienes (cys-LT), which are far more potent as bronchoconstrictor mediators. Alternatively, and more probably, inhibition of COX-1 may reduce tissue levels of a prostaglandin,

possibly PGE₂ that normally suppresses or 'brakes' leukotriene synthesis in the same cell or neighbouring ones. Neither theory explains why a NSAID which inhibits COX-1 in all subjects triggers acute synthesis of cys-LTs only in AERD patients. That the acute triggering of cys-LT synthesis is functionally important in adverse NSAID reactions is nevertheless established by studies with leukotriene synthesis inhibitors and cys-LT receptor antagonists [4]. As well as being potent bronchoconstrictors, the cys-LTs, namely LTC₄ and its extracellular metabolites LTD₄ and LTE₄, are mediators of mucus secretion, microvascular oedema and eosinophil recruitment. LTC₄ is synthesized in mast cells, eosinophils, basophils and monocyte-macrophages because they express the distal enzyme, LTC₄ synthase, an integral nuclear membrane protein belonging to a superfamily of proteins involved in arachidonate metabolism [5].

The most consistent pathological anomaly described in AERD is the finding that even in the absence of NSAID ingestion, the levels of cys-LTs are constitutively elevated in the bronchoalveolar lavage (BAL) fluid, induced sputum, exhaled breath condensate, nasal secretions, saliva, blood and urine of AERD patients compared with aspirin-tolerant individuals [4, 6]. In 1998, my laboratory in Southampton, in collaboration with those of Andrzej Szczeklik in Krakow and K. Frank Austen in Boston, reported that the bronchial biopsies of AERD patients showed fivefold higher counts of cells immunopositive for LTC₄ synthase than those of aspirin-tolerant asthmatics (ATA), and nearly 20-fold more than normal subjects [7]. LTC₄ synthase was expressed in mast cells and macrophages, but the marked increase in AERD subjects was mainly within a greatly expanded population of eosinophils expressing the enzyme. The total numbers of bronchial LTC₄ synthase-positive cells showed striking relationships with the levels of cys-LTs released into BAL fluid in response to local NSAID challenge, and with bronchial sensitivity to an inhaled NSAID, the defining characteristic of aspirin sensitivity.

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We therefore proposed a model for aspirin intolerance [7, 8] in which enhanced bronchial expression of LTC₄ synthase in mast cells, macrophages and eosinophils provides the enzymatic motor that drives constitutive over-production of cys-LTs in AERD subjects. It could also explain why only AERD subjects, and not aspirin-tolerant subjects, produce a further, acute surge in cys-LT production when exposed to NSAIDs; we argued that whether the NSAID causes the shunting of arachidonate or the removal of a PGE₂ brake, only the AERD airway has sufficient LTC₄ synthase-positive cells to generate physiologically-important amounts of cys-LTs in response. The theory that constitutive LTC₄ synthase over-expression is a systemic hallmark of AERD is supported by its demonstration in nasal polyps from AERD patients, associated principally with eosinophilia [9], by increased LTC₄ synthase mRNA in blood eosinophils of AERD patients [10] and by increased LTC₄ generation in cultured mast cells derived from AERD blood monocytes [11].

The LTC₄ synthase hypothesis is also supported by the animal model described in this issue by Hirata et al. [12] from Dokkyo and Chiba universities in Tochigi, Japan. They report that transgenic mice that over-express LTC₄ synthase develop the key pathophysiological features of NSAID intolerance. Hirata and colleagues generated LTC₄ synthase transgenic (LTC4S-Tg) mice with the pCAGGS vector, under the control of a well-described enhancer-promoter sequence. They then immunized LTC4S-Tg and wild-type mice with intraperitoneal ovalbumin (OVA) on days 0 and 5 before inhalation of OVA or saline solution on day 12. This produced three main differences 24–48 h after allergen challenge compared with the wild-type mice. Firstly, the LTC4S-Tg mice showed exaggerated LTC₄ production in their spleen mast cells and in their BAL fluid eosinophils and macrophages *in vitro*. Secondly, this was accompanied by much higher BAL fluid levels of the Th2 cytokines IL-4, IL-5 and IL-13 and by higher eosinophil and lymphocyte counts. Thirdly, the LTC4S-Tg mice gained an 'aspirin-sensitive' phenotype, in which the NSAID sulpyrine greatly enhanced LTC₄ production both *in vitro* and *in vivo* and doubled the OVA-induced rise in airway reactivity, a response that was blocked by a selective CysLT₁ receptor antagonist, pranlukast.

The findings of Hirata et al. [12] are intriguing and raise significant further questions. After immunization and antigen challenge, the transgenic mice displayed LTC₄ production in mast cells, eosinophils and macrophages, but not in T lymphocytes or neutrophils; this matches the pattern of expression of LTC₄ synthase in bronchial and nasal tissues in AERD patients [7, 9]. The increments in *ex vivo* LTC₄ production in the LTC4S-Tg mice were substantial, but broadly comparable with the three- to sevenfold enhanced constitutive production of cys-LTs detected *in vivo* in AERD patients. Associated with the transgenic over-activity of LTC₄ synthase was the recruitment of T

lymphocytes, which may be the cellular source of the remarkably elevated BAL fluid concentrations of Th2 cytokines in the transgenic animals. Increased IL-5 immunoexpression has been noted in AERD bronchial biopsies compared with aspirin-tolerant subjects [7]. Eosinophilopoietic cytokines including IL-5 may act at their cell-surface receptors, along with cys-LTs at the CysLT₁ receptor, to promote eosinophil recruitment and enhance their survival. This in itself would increase the population of cells within the relevant tissue that express LTC₄ synthase and its upstream enzymes. In addition, IL-4, IL-5 and IL-13 may directly upregulate LTC₄ synthase and/or CysLT₁ receptor expression in mast cells, eosinophils and other cells [13–15], while cys-LTs in turn induce IL-4 expression in eosinophils [16]. This may create a vicious circle of eosinophil recruitment, expression of LTC₄ synthase and CysLT₁ receptors, and generation of cys-LTs and Th2 cytokines. Upregulation of CysLT₁ receptors on leucocytes has been reported within the nasal airways in AERD [17] and a similar process on airway smooth muscle cells might promote bronchial reactivity to cys-LTs.

The Hirata paper [12] does not identify the mechanism by which sulpyrine triggered the surge in LTC₄ production in the mixed BAL cells or the increases in BAL fluid LTC₄ levels in the transgenic mice. PGE₂ generated by one or more airway cell-types may operate at one or more EP receptor sub-types to negatively phosphoregulate LTC₄ synthase activity [18] or reduce 5-LO nuclear translocation [19]. Inhibition of PGE₂ synthesis by NSAIDs could therefore liberate the 5-LO/LTC₄ synthase pathway from suppression. The LTC4S-Tg mouse will be useful to define these trigger mechanisms. Administering inhaled PGE₂ before the inhaled sulpyrine should abolish the LTC₄ response in BAL fluid by restoring the braking effect; it should not alter the LTC₄ response to sulpyrine if arachidonate shunting is involved. This experiment would be analogous to the clinical study that implicated PGE₂ braking *in vivo* in AERD patients [20]. Studies in AERD patients undergoing NSAID challenges show concomitant release of a range of cell activation markers including tryptase, histamine and eosinophil cationic protein, suggesting that several cell types may contribute to the aggregate LTC₄ generation. In the Hirata study [12], there is little support from the BAL fluid histamine or PGD₂ data to support the notion that NSAIDs selectively stimulate mast cell activation in AERD individuals by a mechanism independent of COX inhibition [6], at least in this model. Experiments on the isolated BAL leucocyte and spleen mast cell preparations could identify whether a single cell-type is the source of both the PGE₂ brake and the LTC₄ that is generated in response to NSAIDs, or whether intercellular or transcellular processes are required.

While inhibition of PGE₂ is thought to underlie the triggering of acute LTC₄ release, the additional question arises of whether an anomaly or defect in PGE₂ braking

mechanisms is needed to explain why this occurs to a significant extent only in AERD patients. The putative defect could be a deficiency in PGE₂ synthesis or a failure in EP receptor signalling. However, measurements of PGE₂ in nasal secretions, exhaled breath condensate and BAL fluid do not show consistent differences between AERD and aspirin-tolerant patients (reviewed in [4]). A study of urinary metabolites confirmed that global PGE₂ synthesis is not different in AERD and ATA, although paradoxically it was more sensitive to aspirin inhibition in the latter group than in AERD [21]. Nasal or bronchial aspirin challenges nevertheless reduce local levels of PGE₂ equieffectively in aspirin-sensitive and aspirin-tolerant patients [22, 23]. Studies of the expression of the COX isozymes and of the EP1, EP2, EP3 and EP4 receptor subtypes in aspirin-sensitive bronchial and nasal airways similarly produce negative or conflicting results (reviewed in [4]).

In the Hirata study [12], BAL fluid levels of PGE₂ and PGD₂ were similar in LTC₄S-Tg mice to those in the wild-type mice, and they were suppressed equally by inhaled sulpyrine in both groups of animals. This confirms the hypothesis that over-expression of LTC₄ synthase alone, whether in resident cells or in recruited leucocytes, can provide the motive power for an acute LTC₄ response when a uniform PGE₂ brake is removed by the NSAID [8]. In the wild-type mice, the low expression of LTC₄ synthase is inadequate to drive significant synthesis of LTC₄ when an identical PGE₂ brake is removed. An analogy may be drawn with a motor race: the winning car will probably be

the one with the most powerful engine, rather than the one with the most defective brake. While the transgenic LTC₄S mouse model cannot exclude the possibility of a systemic abnormality in the metabolism or activity of prostanoids in clinical AERD, it raises the distinct prospect that such a hypothesis may be redundant.

At the least, the Hirata study should help to focus research effort on the mechanisms that dysregulate LTC₄ synthase in the AERD airway. Hirata et al. [12] provide relatively little information on the baseline characteristics of the LTC₄S-Tg mice, but the requirement of allergic sensitization and challenge for the aspirin-sensitive phenotype to emerge is clearly a puzzle. Many AERD patients have a history of allergic disease to common aeroallergens before becoming sensitive to NSAIDs, but AERD can also emerge *de novo*. Some unknown factor is presumably required to mobilize or activate the intracellular components of the 5-LO/LTC₄ synthase pathway. Early work suggesting that a single nucleotide polymorphism may account for LTC₄ synthase over-expression in AERD [24] has not been sustained in later studies [25]. LTC₄ synthase lies at the heart of a complex circuit of cytokine and cellular interactions in AERD and it may prove difficult to define the precipitating events, but clues may well be sought in the unusual natural history of the syndrome in which viral or staphylococcal infections have been postulated as causative factors [1]. The recent recognition that LTC₄ synthase can be phosphoregulated by Toll-like receptor ligands [18] may focus more attention on chronic infection as an aetiological factor in AERD.

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