

Arachidonic acid metabolism in inflammatory cells of patients with bronchial asthma

Over the last few years, the demonstration of beneficial effects of leukotriene receptor antagonists in various forms of asthma has renewed clinical and pharmacologic interest in this class of lipid mediators. Several studies demonstrated an increased biosynthesis of cysteinyl leukotrienes (CysLT) in asthmatic patients. However, the reasons for the dysregulated production of CysLTs in asthmatic patients are not completely defined. An improved method of lipid mediator detection and the availability of cells isolated from human airways (by bronchoalveolar lavage [BAL] and bronchial biopsies) have allowed initial studies to address this issue. Eosinophils retrieved from inflamed airways of asthmatics have a larger arachidonic acid (AA) content than their blood counterpart. The high level of AA in these cells is primarily due to a remodeling of endogenous arachidonate pools with the accumulation of this fatty acid in a triglyceride-associated pool. In addition, elevated levels of a secretory form of phospholipase A₂, the key enzyme initiating the cascade of CysLTs, are found in the BAL of asthmatics. Finally, eosinophils isolated from the BAL of asthmatics have an increased expression of LTC₄ synthase. The level of expression of this enzyme correlates with the increased amount of CysLTs produced in the airways of these patients. Taken together, these data identify at least two possible mechanisms to explain the excessive CysLT production in asthmatics:

- 1) an increased content of AA in the glycerolipid pools of inflammatory cells
- 2) an enhanced activity of key biosynthetic enzymes involved in CysLT synthesis.

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Several clinical and experimental studies have emphasized the role of chronic inflammation in the pathogenesis of bronchial asthma. A large number of inflammatory mediators, including cytokines, lipid mediators, and enzymes, participate in the development of bronchial asthma, playing a role, often redundant, in the recruitment of inflammatory cells into the airways, and inducing bronchoconstriction and epithelium damage. Over the last few years, the results of clinical trials on leukotriene receptor antagonists in various forms of asthma have renewed the clinical and pharmacologic interest in this class of lipid mediators (1, 2). Furthermore, the introduction of bronchoalveolar lavage (BAL) and transbronchial biopsy procedures in patients with asthma, coupled with improved molecular and cellular biology techniques, has substantially advanced our knowledge of the biochemistry of lipid mediators. Arachidonic acid (AA) represents only 5–10% of esterified fatty acids of glycerolipids in human inflammatory cells, but it constitutes the only

fatty acid capable of generating molecules (eicosanoids) that modulate, in a complex fashion, inflammatory and immune responses (3). Among eicosanoids, the cysteinyl leukotrienes (CysLTs) probably represent the most important class of molecules in the pathogenesis of bronchial asthma (2). The initial step in the biosynthesis of CysLTs (LTC₄, LTD₄, and LTE₄) is the mobilization of arachidonic acid from cellular glycerolipids by various phospholipases A₂ (PLA₂) (4). Arachidonic acid is then transformed into the intermediate hydroperoxide 5-HPETE and, subsequently, into LTA₄ by the same enzyme, 5-lipoxygenase (5-LO). LTA₄ is rapidly converted to LTC₄ in cells containing the enzyme LTC₄ synthase (predominantly mast cells, basophils, and eosinophils in man) (5). LTC₄ is the first active metabolite in the CysLT cascade and the only one synthesized and secreted by inflammatory cells. In the extracellular environment, LTC₄ is subsequently converted to LTD₄ and LTE₄ by a γ -glutamyl transpeptidase and a dipeptidase, respectively (6).

A large number of *in vitro* and *in vivo* studies have demonstrated an increased biosynthesis of CysLTs in asthmatic patients (2, 6, 7). The dysregulated production of these mediators in asthmatics is often coupled with an increased sensitivity of bronchial smooth muscle to the bronchoconstrictor activity of these molecules (8). These observations raised important questions on the regulation of CysLT synthesis in patients with asthma. Answers to some of these questions have been obtained in recent years by studies indicating that asthmatic patients have

- 1) an increased content of AA in the glycerolipid pools of inflammatory cells
- 2) an enhanced activity of key biosynthetic enzymes involved in CysLT synthesis.

Arachidonic acid (AA) pools in inflammatory cells of asthmatic patients

The possibility of comparing cells in a relatively resting state (isolated from blood) with those retrieved in the BAL in asthmatic patients offered great opportunities to explore changes in lipid mediator synthesis induced by the cells' recruitment in an inflammatory area. These studies have shown that some classes and subclasses of membrane phospholipids represent a preferential substrate for PLA₂. In other words, only certain intracellular pools of AA are involved in the production of CysLTs. A linear correlation between the amount of precursors (certain phospholipid pools) and products (eicosanoids) has been demonstrated in several inflammatory cells such as neutrophils, eosinophils, macrophages, and mast cells (4, 9). In particular, only AA derived from the 1-alkyl subclasses of phosphatidylcholine and phosphatidylethanolamine has been closely linked to eicosanoid synthesis. In contrast, AA derived from other classes of phospholipids (phosphatidylinositol and phosphatidylserine) or from the 1-acyl subclass of phosphatidylcholine and phosphatidylethanolamine seems to be preferentially secreted as free AA or to remain in cells, acting as a second messenger.

In macrophages obtained from human lung parenchyma, we have recently identified a new pool of AA esterified in triglycerides (TG) (10). This pool contains 25% of the total intracellular AA in macrophages. Subsequent studies have shown some unique features of the AA pool associated with TG. AA incorporated by the cell is initially esterified into the TG pool and subsequently transferred into the phospholipid pools. The amount of AA esterified into TG correlates significantly with the total cellular content of AA; i.e., the larger the quantity of AA within the cell, the higher the percentage of AA associated with TG (11). The

reason for this phenomenon is that the TG pool is a large, rapidly expandable pool for AA (high capacity, low affinity). In contrast, the phospholipid pools have a relatively low capacity to esterify AA (high affinity, low capacity) (12). We have recently extended these observations, showing that the AA pool associated with the TG is constitutively present in many human inflammatory cells but at different levels. In human lung mast cells, for example, the TG pool contains approximately 50% of the total cellular AA, whereas this pool contains 10% of cellular AA in monocytes and platelets. In resting neutrophils and eosinophils, only 3% of the total content of AA is associated with TG. However, when the eosinophils were isolated from patients with hypereosinophilic syndromes, they were found to contain up to 20% of AA esterified into TG (12).

This observation suggested that in inflammatory cells, such as eosinophils and neutrophils recruited in the lung during an inflammatory response, there might be a remodeling of AA pools, with an increase of AA associated with the TG pool. This hypothesis was confirmed by showing that cells recovered from the BAL of patients with adult respiratory distress syndrome (neutrophils) or with bronchial asthma (eosinophils) contain an AA pool in TG 4–10 times larger than that stored in the cells isolated from the peripheral blood of these patients (12). Subsequent studies have demonstrated that the pool of AA associated with TG is located in lipid bodies, non-membrane-bound cytoplasmic organelles whose function is still unknown. Lipid bodies are abundant in human lung macrophages and mast cells, but they are very few in resting neutrophils and eosinophils (13). However, there is a dramatic increase in the number of lipid bodies in eosinophils recovered from the BAL of asthmatic patients. In these cells, the increase in the number of lipid bodies correlates significantly with the accumulation of AA in the TG pool. Interestingly, the remodeling of AA pools observed *in vivo* in eosinophils recruited in the airways of asthmatic patients could be reproduced *in vitro* by incubating normal eosinophils with GM-CSF for 24 h in the presence of exogenous AA (12). In these experimental conditions, eosinophils acquire the morphologic features of "hypodense" cells, similar to those found in BAL eosinophils and macrophages of asthmatic patients (14).

Taken together, these observations suggest that a large increase in the amount of AA occurs in cells recruited in an inflammatory area, such as the eosinophils in the lungs of asthmatics. This increase is mostly due to the expansion of the TG pool, which functions as a large reservoir of AA to be later transferred to the phospholipid pools. The accumulation of AA in TG in the eosinophils of asthmatics ultimately leads to an increased substrate availability for the synthesis of CysLTs in these cells.

Activation of enzymes involved in CysLT synthesis

PLA₂ is an enzyme that hydrolyzes AA from phospholipids, and it represents the key enzyme in the synthesis of eicosanoids. Different isoenzymes of PLA₂ have been identified in human inflammatory cells: the classical cytosolic PLA₂ (cPLA₂; group IV), at least two secretory PLA₂s (sPLA₂; groups IIa and V), and a Ca²⁺-independent PLA₂ (group VI). In 1989, Kramer et al. reported the isolation and characterization of an extracellular, low-molecular-mass (14 kDa) PLA₂ in the synovial fluid of patients with rheumatoid arthritis (15). This enzyme shares common features with the PLA₂ secreted by thrombin-activated human platelets and with those present in snake venoms (PLA₂ group I). Briefly, secretory PLA₂ is activated only by millimolar concentrations of Ca²⁺, it contains a large number of disulfide bridges, and it recognizes all types of fatty acids at the sn-2 position of phospholipids (16). Clinical studies have shown that large amounts of sPLA₂ are released in the plasma of patients with systemic anaphylaxis and in the nasal lavage of patients with allergic rhinitis after specific antigen challenge (17, 18). We have recently demonstrated that BAL obtained from patients with mild persistent asthma contains levels of sPLA₂ fivefold higher than that obtained from normal subjects (19). Biochemical and molecular characterization revealed that the enzyme secreted in the BAL was group IIA sPLA₂. A major cellular source of sPLA₂ released in the BAL was found to be the mast cell. In fact, human mast cells isolated from the lung parenchyma and activated by immunologic stimuli release sPLA₂ identical to that found in the BAL of asthmatic patients (20).

The role of the sPLA₂ released in the alveolar fluid in the pathogenesis of bronchial asthma is still to be determined. However, initial evidence indicates that this enzyme can activate a number of inflammatory cells, including lung mast cells, macrophages, and eosinophils, by interacting with a specific membrane receptor (21). Therefore, sPLA₂ released in the airways of asthmatics might function as a proinflammatory mediator by at least two mechanisms:

- 1) by releasing AA from surfactant phospholipids
- 2) by directly recruiting inflammatory cells in the airways.

The first enzyme-generating molecule committed to the CysLT cascade is LTC₄ synthase. Sequencing and cloning of LTC₄ synthase was achieved in early 1990, opening interesting ways to explain the enhanced synthesis of CysLTs in asthmatic patients (22). The human gene for LTC₄ synthase is located on chromo-

some 5 in a DNA region containing other genes relevant to atopic diseases, including those for IL-3, IL-4, IL-5, GM-CSF, and the β_2 -adrenergic receptor. The clustering of genes important for the development of allergic diseases suggests that common regulatory mechanisms may be operating in the control of their transcription. Recent studies have shown an increased expression of LTC₄ synthase in the lung of patients with aspirin-induced asthma (7). This particular form of asthma has long been associated with an increased generation of CysLTs *in vitro* and *in vivo*. The overexpression of LTC₄ synthase provides a molecular basis for those observations. This finding has been recently extended to bronchial biopsies of patients with allergic asthma and to nasal biopsies of patients with allergic rhinitis. LTC₄ synthase was the only enzyme involved in eicosanoid synthesis to be overexpressed; i.e., there was no enhanced expression of cPLA₂, 5-lipoxygenase, or cyclooxygenases 1 and 2. Immunohistochemical analysis showed that the only cells overexpressing LTC₄ synthase in both allergic and aspirin-induced asthmatics were eosinophils, and not, for example, mast cells, macrophages, or epithelial cells (7).

Conclusion

There is general agreement that an increased production of CysLTs occurs in subsets of patients with various forms of bronchial asthma. Major efforts are currently devoted to understanding the molecular and biochemical basis for CysLTs overproduction in these patients.

Two mechanisms have been identified so far:

- 1) an increased accumulation of AA, the substrate for CysLTs, in cells recruited in asthmatic airways (mostly eosinophils)
- 2) an enhanced activity and release of sPLA₂ and of LTC₄ synthase.

Both these mechanisms are consistent with the increased production of CysLTs, at least in a group of asthmatics. A crucial role is played by eosinophils, in which both mechanisms have been demonstrated. Evaluation of these two parameters, AA storage and enzyme expression, can be easily done in cells recovered from BAL. On a practical basis, this approach can be used to identify patients who will respond to antileukotriene therapy with CysLTs-receptor antagonists (montelukast or zafirlukast). However, these studies are still underway, and their results will be useful not only to expand our knowledge on the pathogenesis of asthma, but also to identify individuals at risk of developing atopic diseases.

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