Imbalance Between Leukotriene Synthesis and Catabolism Contributes to the Pathogenesis of Allergic Diseases

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Abstract: Recently, there has been great progress in elucidating the biochemistry of the arachidonic acid (AA) metabolism. The abnormal production of leukotrienes (LTs), due to the unbalanced-regulation of synthesizing and catabolizing enzymes, is probably induced by many bioactive substances, including Th2 cell-derived cytokines. Imbalances in these processes may play an important role in allergic reactions and other inflammatory diseases, thus making them potentially prime therapeutic targets for these diseases. Further studies on the regulation of LT-synthesis and catabolism are therefore required to clarify the importance of these imbalances on the initiation and progression of allergic and inflammatory diseases.

INTRODUCTION

Leukotrienes (LTs; LTC_4, LTD_4, LTE_4, and LTB_4), metabolites of arachidonate-5-lipoxygenase (5-LO) pathway [1], are produced in mast cells, basophils, eosinophils and neutrophils as part of the process of allergic inflammation. The cysteinyl LTs (cysLTs; LTC_4, LTD_4 and LTE_4) are particularly potent stimulators of mucus secretion, bronchial hyperresponsiveness, constriction of the airway smooth muscle, and increased vascular permeability [2-5], which can contribute to asthma attack in humans [6]. This may explain why cysLT-receptor antagonists are therapeutically effective for bronchial asthma [7-9].

The synthesis of LT is initiated by the transmembranous stimulation due to various factors which increase the level of intracellular calcium ion, thus leading to the translocation of cytosolic phospholipase A_2 ($cPLA_2$) to the nuclear membrane. $cPLA_2$ cleaves membrane phospholipid to form arachidonic acid (AA). The released AA is converted into LTA_4 *via* an unstable intermediate, 5-hydroperoxyeicosatetraenoic acid (5-HPETE), by 5-LO, which is also translocated from the cytosol to the nuclear membrane where five-lipoxygenase activating protein (FLAP) resides. The next step is the transformation of LTA_4 to LTC_4 by LTC_4 synthase, and to LTB_4 by LTA_4 hydrolase. The newly synthesized LTC_4 and LTB_4 are released outside the cells. LTC_4 is enzymatically metabolized to LTE_4 *via* LTD_4 by two consecutive enzyme families, namely glutamyl transpeptidases (GTPs) and dipeptidases. Recent studies demonstrate that LTs-synthesis and catabolism are regulated by stimulation with various bioactive substances (Fig. **1**).

We herein review the recent findings on LT-synthesizing and catabolizing enzymes in the context of those from other investigators. These findings are related to the regulatory mechanisms associated with allergic diseases.

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REGULATION OF LTS SYNTHESIZING ENZYMES

Phospholipase A_2

PLA_2 family consists of several different enzymes (Type I, II A, II C, V, IV and Ca^{2+} independent enzymes). A recent study demonstrated $cPLA_2$ (type IV) to be activated and translocated to the perinuclear membrane by an increase in the level of intracellular calcium ion to the micromolar level. $cPLA_2$ is the enzyme predominantly responsible for receptor-mediated increases in AA metabolism. Several investigators have reported the enzyme activity of $cPLA_2$ to also be induced by various growth factors, interleukins (ILs) and other bioactive substances [10,11]. We also previously reported $cPLA_2$ to be transcriptionally up-regulated by lipopolysaccharide (LPS) in human polymorphonuclear leukocytes (PMNs) [12]. A recent study confirmed that an innate system response, toll like receptor (TLR) 4 signaling, regulates $cPLA_2$ in LPS stimulated macrophages [13].

Five-Lipoxigenase and Five-Lipoxygenase Activating Protein

5-LO catalyzes the conversion of AA to 5-HPETE, and further to LTA_4 in the presence of FLAP, an arachidonate binding protein. 5-LO plays a central role in LT biosynthesis. The expression of 5-LO and FLAP are also induced by granulocyte-macrophage colony-stimulating factor (GM-CSF) [14,15]. Cowburn *et al*. demonstrated that IL-5, a T helper type 2 (Th2) cell-derived cytokine, increases the expression of 5-lipoxygenase-activating protein [16]. In addition, we also recently confirmed that IL-18, which is an important cytokine in viral infection, increased the 5-LO expression in human mast cells [17].

LTC_4 Synthase

Human LTC_4 synthase is a glutathione-S-transferase specific for LTA_4. The LTC_4 synthase gene has been cloned and mapped to chromosome in 5q35, distal to that of cytokines (IL-3, IL-5 and GM-CSF), which all play an important role in the regulation of allergic inflammation. The up-regulation of LTC_4 synthase has been demonstrated in a few reports. Recently, Hsieh *et al*. demonstrated that IL-4 dramatically

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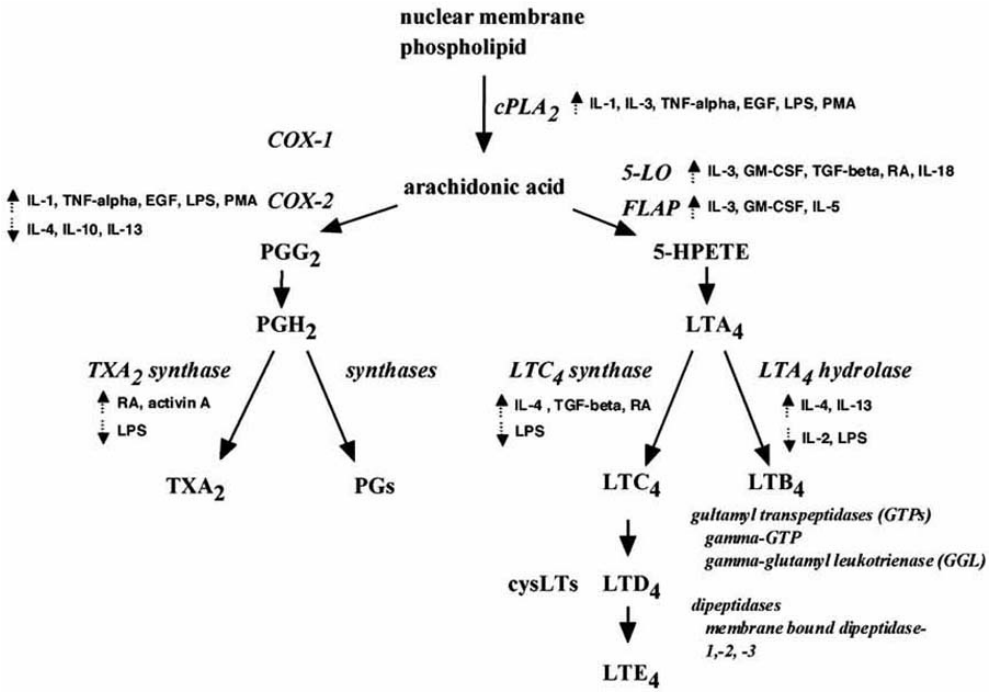

Fig. (1). Metabolism of arachidonic acid; bioactive substances regulate the metabolizing enzymes.

Activated $cPLA_2$ liberates arachidonic acid from membrane phospholipids. Free arachidonic acid is converted to PGH_2 by COX. PGH_2 is metabolized to PGs by PG synthases or to TXA_2 by TXA_2 synthase. On the other hand, 5-LO, translocated to the nuclear envelope where FLAP resides, converts arachidonic acid to LTA_4 *via* 5-HPETE. LTA_4 is metabolized to LTC_4 by LTC_4 synthase or to LTB_4 by LTA_4 hydrolase. Recently, investigators have demonstrated that the arachidonic acid-metabolizing enzymes are regulated by various agents including interleukins. PG, prostaglandin; LT, leukotriene; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; $cPLA_2$, cytosolic phospholipase A_2; COX, cyclooxygenase; 5-LO, 5-lipoxygenase; FLAP, 5-lipoxygenase activating protein; IL, interleukin; TNF-alpha, tumor necrosis factor-alpha; EGF, epidermal growth factor; LPS, lipopolysaccharide; PMA, phorbol 12-myristate 13-acetate; RA, retinoic acid; GM-CSF, granulocyte-macrophage colony-stimulating factor; TGF-beta, transforming growth factor-beta.

induced the expression of LTC_4 synthase mRNA and protein in stem cell factor (SCF)-primed human mast cells, derived from cord blood mononuclear cells [18]. On the other hand, Serio *et al*. reported that LPS down-regulates LTC_4 synthase gene expression in a cell-specific manner in the monocyte-like cell line, THP-1 [19].

LTA_4 Hydrolase

LTA_4 hydrolase catalyzes the most distal step in the production of LTB_4. LTB_4 is a potent chemotactic factor for neutrophils and eosinophils, thus stimulating their adherence to endothelial cells, degranulation and generation of reactive oxygen species. Recently, Jozefowski *et al*. demonstrated that LTB_4 modulates IL-12 release from dendritic cells, thereby indicating the T helper 2-polarizing effects of leukotrienes [20]. LTA_4 hydrolase has also been purified from a variety of sources. We recently found that IL-4 and IL-13 up-regulate LTB_4 synthesis in PMNs by increasing the LTA_4 hydrolase expression, although LPS and IL-2 down-regulate LTB_4 synthesis by decreasing the LTA_4 hydrolase expression [21]. This is a novel regulatory mechanism of the AA metabolism in human PMNs and it probably also plays an important role in the pathogenesis of bronchial asthma. Because, IL-4 and IL-13 are Th2 cell-derived cytokines, which are predominantly produced in excess in allergic patients, they are considered to play major roles in the pathophysiology of allergic diseases. We therefore speculate that the level of LTA_4 hydrolase expression increases during allergic reaction by the stimulation of Th2 cell-derived cytokines, while it decreases by the stimulation of Th1-derived cytokines.

REGULATION OF LTS CATABOLIZING ENZYME

Glutamyltranspeptidases and Dipeptidases

The regulation of the individual's steps in LT synthesis has been examined in several studies. However, so far very few studies have investigated the catabolism of LTs. LTC_4 is enzymatically metabolized to less active LTE_4 *via* LTD_4 by GTPs and dipeptidases. It had been considered that gamma-GTP was the only enzyme to mediate the conversion of LTC_4 to LTD_4. Recently, another enzyme showing a gamma-GTP like activity, named gamma-glutamyl leukotrienase (GGL), was identified in humans and mice [22,23]. Similar findings have also been demonstrated in dipeptidases, thus suggesting that there are families of enzymes that have the same or similar catabolic activities.

We previously reported that the abundance of mRNA for GGL but not for gamma-GTP was up-regulated by the treatment of cells with glucocorticosteroids (GCSs), such as fluticasone propionate (FP) and beclomethasone dipropionate

(BDP), which are associated with an increased LTC_4-degrading activity [24]. We considered the regular use of FP and BDP to up-regulate the conversion of LTC_4 to LTE_4, therefore contributing to its anti-asthma actions. Although the inhibition of Th2 cytokine production by GCSs is a widely accepted mechanism in the treatment of asthma, the accelerated conversion rate of LTC_4 to LTE_4 in patients with asthma may also be an important component of GCSs.

THE BIOLOGICAL ROLE OF LEUKOTRIENES

As previously reported, cysLTs are potent bronchoconstrictors, 100-1,000 times more potent than histamine [1,2]. Asthmatic subjects appear to be more sensitive to inhalational challenge with LTE_4. Inhalation of LTE_4 has been shown to lead to increased numbers of eosinophils into lungs of asthmatics [25]. In addition, recent study suggests that cysLTs are important in prolonging eosinophil survival. CysLTs have been shown to be potent mucus secretion [26]. CysLTs also contract vascular smooth muscle, thus contributing to extra vascular leakage [27]. A recent study revealed that cysLTs also play an important role in the immune and inflammatory responses [28].

LTB_4 is a potent chemoattractant for neutrophils and eosinophils, as well as an activator of these cells which appears to enhance the adhesion and migration of cells throughout the endothelium [29].

THE LEVELS OF LTS IN BIOLOGIC FLUIDS IN PATIENTS

CysLTs and LTB_4 have been measured in the body fluids of asthma patients, including bronchoalveolar lavage (BAL) fluid, urine and blood [30, 31]. The cysteinyl LTs are known to increase in the BAL fluid of asthmatics after allergen and aspirin challenges [30]. They are also present in nasal fluid following allergen challenge, and in the setting of respiratory syncytial virus infection [32]. Several reports have recently demonstrated that cysLTs can be detected in the exhaled breath condensate (EBC), with elevated levels reported in adults and children with asthma [33, 34].

We examined the conversion rate of LTC_4 to LTE_4 in the serum from children with asthma, and compared them with that in the controls. The conversion rate of LTC_4 to LTE_4 was significantly lower in children with asthma [35].

DISCUSSION

The increased production of prostaglandins (PGs) and LTs has been considered to be an important finding in allergic and inflammatory diseases [6]. One of the mechanisms of the enhanced production is the induction of new synthesizing enzymes. We previously reported that the mRNA-expression for 5-LO, LTA_4 hydrolase and LTC_4 synthase was up-regulated in the peripheral blood leukocytes from children with bronchial asthma [36]. The up-regulated enzymes play important roles in allergic and inflammatory diseases, and the inhibition of those may thus be therapeutic targets for patients with these diseases.

Another mechanism for the increasing the levels of LTs in human tissue specimens may be by decreasing their catabolism. We previously demonstrated that the decreased conversion rate of LTC_4 to LTE_4 might be important in the pathogenesis of childhood asthma [35]. The immediate degrading of LTC_4 to LTE_4 may attenuate such asthma exacerbations. The detection of gamma-GTP and dipeptidases families has been progressing. The regulation of these enzymes in allergic and inflammatory diseases may also be mediated by bioactive substances including cytokines.

As shown in Fig. (**1**), we and others have suggested that LT synthesis increases in the process of allergic reaction by the stimulation of Th2 cell-derived cytokines, which enhances allergic inflammation. Hsieh *et al*. demonstrated that IL-4 dramatically enhanced the cysLT production in stem cell factor (SCF)-primed human mast cells, derived from cord blood mononuclear cells [18] Recently, Jiang *et al*. also confirmed that IL-4 induced intracrine cysLT synthesis [37]. We also reported that IL-4 and IL-13 up-regulate LTB_4 synthesis in PMNs [21]. On the other hand, LT synthesis decreases during the process of inflammatory reaction by the stimulation of Th1 cell derived cytokines. Serio *et al*. reported that LPS down-regulates cysLT synthesis in a monocyte-like cell line, THP-1 [19]. Recently, Coffey *et al*. reported that LPS and INF-gamma down-regulated the 5LO metabolism in murine alveolar macrophages [38]. We also demonstrated that LPS and IL-2 attenuate the LTB_4 production in human PMNs [21]. Therefore, the unbalanced regulation of LT synthesis by cytokines may play an important role in allergic and inflammatory diseases. Further studies will be required to elucidate this proposition.

Interestingly, a recent study revealed the expression of a receptor for cysLT, cysLT1, to also be regulated by biological stimuli. For example, cysLT1 increases after the stimulation of Th2 cell-derived cytokines, which thus enhances allergic inflammation, such as IL-4 and IL-13 [39,40]. On the other hand, the expression of cysLT1 decreases in the process of an inflammatory reaction by LPS [41]. The imbalanced expression of LT receptors might also play an important role in allergic and inflammatory diseases. The unbalanced regulation of LT synthesis and/or LT receptors by cytokines, especially by Th2 type cytokines, may also play an important role in allergic and inflammatory diseases (Fig. **2**).

Our understanding of the molecular mechanisms and regulation of LT-synthesis has recently been dramatically increasing. The human genes for $cPLA_2$, 5-LO, FLAP, LTC_4 synthase and LTA_4 hydrolase have been cloned and their promoter regions have been shown to be involved transcriptional regulatory mechanism. Sanak *et al*. reported the LTC_4 synthase expression to be up-regulated in patients with aspirin induced asthma, which thus might be genetically determined [42]. In addition, polymorphism of the 5-LO, FLAP, LTC_4 synthase and/or LT receptors gene has also been reported to possibly contribute to the pathogenesis of allergic diseases [43-46].

The imbalance between LT synthesis and catabolism is an important finding in allergic and inflammatory diseases. Further studies on the regulation of LT synthesis and catabolism, especially using *in vivo* studies, will be important for clarifying the pathophysiological roles of these lipid mediators in allergic and inflammatory diseases.

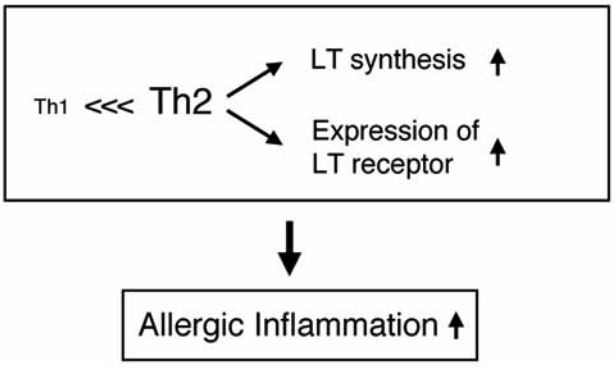

Fig. (2). Unbalanced regulation of LT synthesis and/or LT receptors by cytokines may play an important role in allergic inflammation.

LT synthesis and the expression of cysLT1 increases in the process of allergic reaction by the stimulation of Th2 cell-derived cytokines, which enhances allergic inflammation. LT, leukotriene.

ACKNOWLEDGEMENTS

This work was supported in part by grant from the Ministry of Education, Science, Sports, and Culture of Japan (No. MK17591096).

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Received: 04 September, 2006 **Revised: 28 March, 2007** **Accepted: 02 April, 2007**