Leukotriene receptor antagonists in the treatment of asthma: Implications for eosinophilic inflammation

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Recent advances in the treatment and management of asthma have suggested that leukotriene (LT) receptor antagonists may be very beneficial as a second generation therapy with steroid-sparing properties and negligible side effects. These agents have shown interesting effects on peripheral blood and sputum eosinophils. A major contributor to the damage in the airway of asthmatic patients is the eosinophil, which, upon activation, releases a battery of granule-associated cytotoxic, cationic proteins, including the major basic protein and eosinophil peroxidase, and membrane-derived de novo-synthesized bioactive lipid mediators, including LTC_4, LTD_4 and LTE_4, as well as platelet activating factor. These products have deleterious effects on the airway tissue including mucosal and smooth muscle layers. Accumulating evidence suggests that these agents may also influence the accumulation and maintenance of eosinophilic responses at the site of inflammation. This article reviews the possible anti-inflammatory mode of action of these therapies. It also discusses where there may be a gap in the knowledge regarding the potential direct and indirect effects of LT modifiers on eosinophil function and recruitment.

Key Words: *Asthma; Eosinophil; Inflammation; Leukotriene*

Les antagonistes des récepteurs des leucotriènes dans le traitement de l'asthme : implications pour l'inflammation éosinophile

RÉSUMÉ : Les progrès récents réalisés dans le traitement et la prise en charge de l'asthme laissent croire que les antagonistes des récepteurs des leucotriènes (LT) peuvent être très bénéfiques comme traitement de deuxième génération avec des propriétés permettant de réduire les stéroïdes et des effets secondaires négligeables. Ces agents ont démontré des effets intéressants sur les éosinophiles contenus dans l'expectoration et dans le sang périphérique. Un des principaux responsables des dommages causés aux voies aériennes des patients asthmatiques est l'éosinophile, qui, lorsqu'il est activé, libère une gamme de protéines cationiques, cytotoxiques associées aux granules de l'éosinophile, incluant la protéine majeure basique et la péroxidase de l'éosinophile, et des médiateurs lipidiques bioactifs néoformés et dérivés de la membrane, comprenant les LTC4, LTD4 et LTE4 de même que le PAF. Ces produits ont des effets délétères sur les tissus des voies aériennes y compris sur les couches des muqueuses et du muscle lisse. Un nombre grandissant de preuves permettent de croire que ces agents pourraient aussi influer sur l'accumulation et le maintien des réponses éosinophiles au site de l'inflammation. Le présent article passe en revue le mode d'action antiinflammatoire potentielle de ces traitements. Il discute également des endroits où il peut y avoir des failles dans les connaissances concernant les effets directs et indirects potentiels des modificateurs des LT sur la fonction et le recrutement éosinophile.

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Asthma is a heterogeneous and complex condition afflicting a wide range of population of varying age and sex. It is now generally recognized that the disease is caused mainly by inflammation in the airways (Figure 1). This results in reversible airway obstruction and bronchial hyperresponsiveness, which at times can be fatal. Until recently, the treatment of asthma was confined to symptom relieving (beta$_2$-agonists) and/or preventive therapy, ie, corticosteroids (inhaled and/or systemic).

Activated T cells and eosinophils are thought to play a major role in asthma, and the numbers of these cells correlate broadly with disease severity (1). Mucosal damage in chronic asthma is believed to be the consequence of cytotoxic and proinflammatory mediator release from activated eosinophils (2,3). These include cytotoxic granule proteins (major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil-derived neurotoxin) together with phospholipid-derived, pharmacologically active mediators. Cytokines derived from T helper (Th) 2-type cells, particularly interleukin (IL)-3, IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF), are thought to regulate eosinophil activation and promote their survival (4,5). IL-5 is an obligatory terminal differentiator of eosinophil precursors (6).

SULPHIDOPEPTIDE (CYSTEINYL) LEUKOTRIENES

Eosinophils are a rich source of the sulphidopeptide leukotrienes (2). These are de novo-synthesized lipid mediators derived from arachidonic acid (AA) by the action of phospholipase A$_2$ (PLA$_2$) (7). AA is found in association with the plasma membrane as well as cytoplasmic lipid bodies in these cells (8), and is metabolized through one of two pathways, cyclo-oxygenase and lipoxygenase (9). The former leads to the generation of prostaglandins, thromboxanes and prostacyclins. The second pathway, via the 5-lipoxygenase (5-LO) enzyme acting in concert with a cofactor, 5-LO activating protein (FLAP), generates leukotrienes (LTs), including LTB$_4$, LTC$_4$, LTD$_4$ and LTE$_4$ (10).

The 5-LO enzyme cleaves AA to form 5-hydroperoxy eicosatetranoic acid [5-HPETE] and the subsequent synthesis of an unstable intermediate epoxide (LTA$_4$), which is translocated to the perinuclear membrane (11). In turn, LTA$_4$ rapidly converts to either LTB$_4$ (via the action of LTA$_4$ hydrolase), or the cysteinyl LT LTC$_4$ (5S-hydroxy-6R,S-glutathionyl-7,9,-trans-11,14-cis-eicosatetraenoic acid) through the action of LTC$_4$ synthase in the nuclear membrane. This is achieved by adding the tripeptide glutathione onto LTA$_4$ (7).

Eosinophils produce negligible amounts (6 ng/10^6 cells) of LTB$_4$ (5S-12R-dihydroxy-6,14-cis-8,10-trans-eicosatetraenoic acid) (12) compared with up to 200 ng/10^6 cells from neutrophils. LTB$_4$ is a chemotactic, priming and activating factor for leukocytes, including neutrophils (13). In contrast, human eosinophils generate relatively large quantities of LTC$_4$ (up to 70 ng/10^6 cells) after stimulation with the calcium ionophore A23187 (14). In general, eosinophils obtained from asthmatic subjects appear to produce more LTC$_4$ than those from normal healthy donors (15,16). Furthermore, co-culture of eosinophils with endothelial cells (17), or exogenous addition of cytokines, eg, IL-3, IL-5, GM-CSF and tumour necrosis factor-alpha (TNFα) was shown to result in the upregulation of ionophore-induced release of LTC$_4$ (18-20).

When LTC$_4$ is formed, it is transported actively out of the cell. LTD$_4$ and LTE$_4$ are produced from LTC$_4$ by the removal of glutamic acid (via the action of -glutamyl transpeptidase) and glycine (via the action of dipeptidase), respectively (7,9). LTC$_4$, D$_4$ and E$_4$ collectively form the activity previously recognized as 'slow reacting substance of anaphylaxis', due to their prolonged in time spasmogenic effects on smooth muscles. Although LTD$_4$ and LTE$_4$ are rapidly degraded in the body through oxidative metabolism, small amounts of LTE$_4$ can be measured in the urine (21). Interestingly, in humans, the leukotriene pathway, via 5-LO, is observed only in myeloid cells, ie, mast cells, basophils, neutrophils, eosinophils and alveolar macrophages (22). Recent studies have suggested that cysteinyl LTs may also have selective eosinophilotactic activity (23,24).

LT RECEPTORS

Two receptors for cysteinyl LTs have been identified on smooth muscle cells, namely, Cys-LT1 and Cys-LT2. Cys-LT1 is now recognized as the probable regulating receptor for bronchial smooth muscle contraction and, thus, may be directly relevant to asthma treatment (25). Cys-LT2, on the other hand, appears to be mainly involved in pulmonary vein contraction (26). In addition to LTD$_4$, both LTC$_4$ and LTE$_4$ bind to Cys-LT1, although LTE$_4$ exhibits a greatly reduced binding capacity (25). There is, however, no evidence available in the literature to suggest that eosinophils express either of these two receptors for LTs. One exception is the observation that HL-60 cells, which were differentiated into eosinophils, in vitro, expressed a very low affinity LTD$_4$ receptor (Kd 41.91 nM) (27). However, these cells may not reflect fully all the properties and the biological profile of circulating mature eosinophils.

EVIDENCE FOR A BIOLOGICAL ROLE FOR LTS IN ASTHMA

Evidence has accumulated to suggest that, in vivo, LTs are among the most potent constrictors of airway smooth muscle and are, thus, major players in the complex picture of asthmatic inflammation. A substantial amount of the published literature shows that LTs are critical elements in the development, progression and chronicity of the inflammatory response associated with various clinical presentations of asthma (28-30). For instance, both LTC$_4$ and LTD$_4$, when inhaled, are an order of magnitude greater in pharmacological potency than histamine in inducing airflow obstruction in normal subjects, and their effects lasted longer. In these studies, asthmatic airways appeared to be more sensitive (100 to 1000 times) to inhaled cysteinyl LTs than those of nonasthmatic subjects. These patients also demonstrated increased bronchial hyperresponsiveness to methacholine or histamine following inhalation of LTC$_4$ and LTD$_4$ (31).

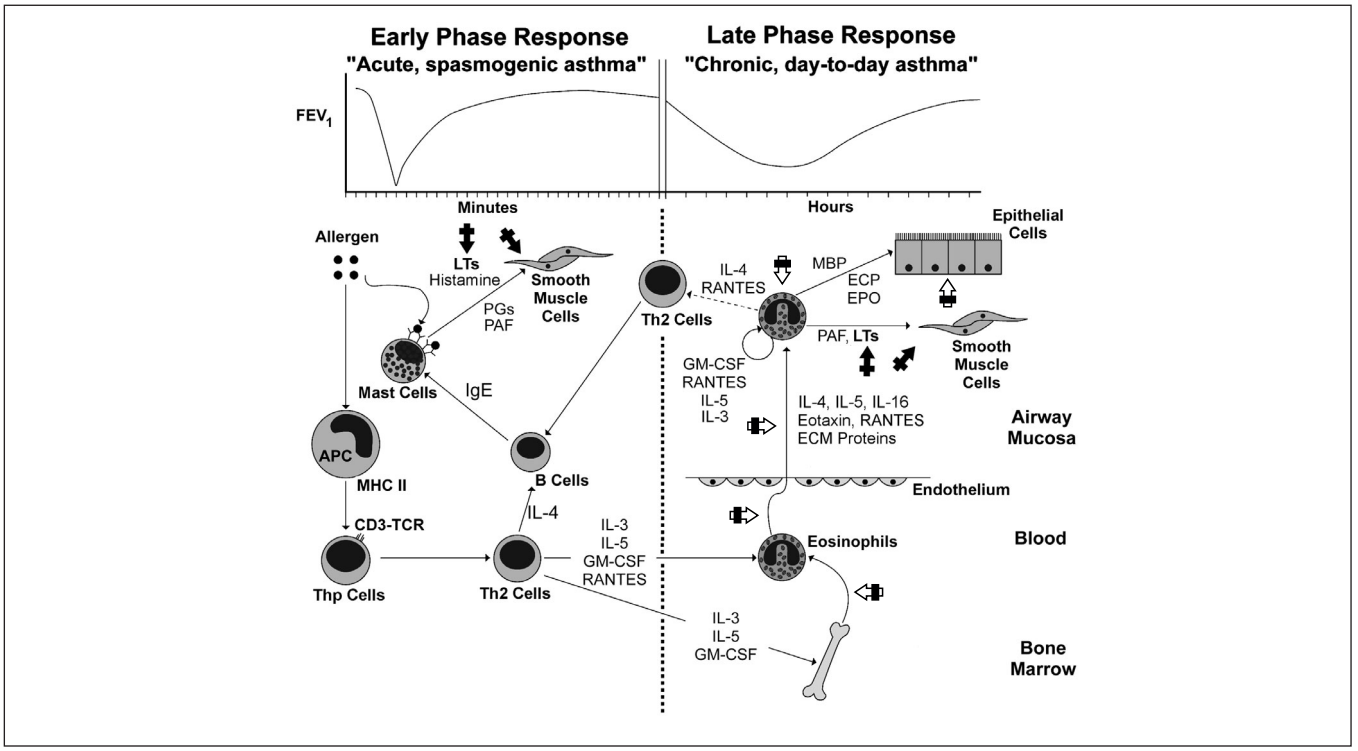

Figure 1) *A schematic representation of the current understanding of putative immune and inflammatory mechanisms regulating the early late phase asthmatic response. Cytokines and chemokines have the potential to exert regulatory influences on the inflammatory cascade involving the eosinophil. Leukotriene (LTs) modifiers are thought to modulate the response ( ) at the level of the smooth muscle cells. Little is known about the potential of these agents to influence eosinophil or epithelial cell responses ( ). APC Antigen presenting cells; ECM Extracellular matrix proteins; ECP Eosinophil cationic protein; EPO Eosinophil peroxidase; FEV_1 Forced expiratory volume in 1 s; GM-CSF Granulocyte-macrophage colony-stimulating factor; Ig Immunoglobulin; IL Interleukin; MBP Major basic protein; MHC Major histocompatibility complex; PAF Platelet activiating factor; PG Prostaglandin; Th T helper cells; Thp T helper precursor; TCR T cell receptor*

LT MODIFIERS

The potential contribution of the sulphidopeptide LTs in bronchoconstriction has been identified by recent developments in the field of LT-modifying therapies (30,32). Earlier studies and trials with LT inhibitors and receptor antagonists were disappointing, mainly due to toxicity, lack of specificity and limited potency of the agents (33).

Two new strategies aimed at blocking the effects of the LT pathway have recently gained rapid momentum and provided hope for better and more efficient treatment for asthma. The first area relates to the development of LT synthesis inhibitors, such as zileuton and BAYx1005. These were shown to produce significant reductions in LT generation levels, including both LTB_4 and the cysteinyl LTs (34,35). Zileuton inhibits the 5-LO and, thus, prevents the synthesis of LTA_4 (34). In contrast, BAYx1005 is an antagonist of FLAP, which prevents the translocation of 5-LO and blocks the formation of LTA_4 (35). This area of FLAP inhibition is currently under intensive development because it will determine the role of other products of 5-LO metabolic pathway in asthmatic inflammation.

More recently, second generation LTD_4 receptor antagonists have been introduced. These new agents have the potential to inhibit the biological activities of LTD_4 and the other members of the cysteinyl LT family by competing for their receptors on smooth muscle cells (36). These include montelukast (Singulair [Merck Frosst Canada Inc, Kirkland, Quebec]), zafirlukast (Accolate [Zeneca Pharma Inc, Mississauga, Ontario]), and pranlukast (Ultair, SmithKline Beecham, United Kingdom), which, when used in inhalation challenge studies, in vivo, have demonstrated a greater potency in blocking LT effects and a better safety record (37-40).

LT MODIFIERS AND THE EOSINOPHIL IN ASTHMA

The clinical effects of LT receptor antagonists (including montelukast and zafirlukast) on dampening the pathophysiological sequelae of inflammation in asthma have been amply reviewed previously (37-41). These reports show clearly that these agents have significant efficacy in controlling asthma symptoms in a large percentage of patients. The present paper concentrates on the as yet unanswered question of whether LT receptor antagonists are anti-inflammatory drugs, potent bronchodilators or both. In particular, the issue of the biological relevance and efficacy of LT receptor antagonists from the perspective of their effect on the eosinophil is addressed. As stated earlier, the latter is a highly relevant inflammatory cell type in asthmatic airway mucosal tissue (Figure 1) (3).

Recent observations have shown that both montelukast and zafirlukast suppress eosinophil numbers in the peripheral

blood and airways (42,43). Using hypertonic saline-induced sputum as an indicator of cellular infiltration associated with asthmatic inflammation, researchers demonstrated significant reductions in the numbers of sputum eosinophils following treatment with montelukast (44). A consequence of such studies has been to provide further fuel to the hypothesis, and possibly a subtle conclusion, that cysteinyl LTs possess eosinophilotactic properties (23,24,42).

There are many areas that have not yet been studied in relation to the effect of LT receptor antagonists on eosinophil biology and its natural history in asthma (Figure 1). In order to reach a better conclusion about the biological effects of leukotriene modifiers on eosinophils in asthma, further supportive evidence is needed. LT activity blockers, such as montelukast and zafirlukast, may indeed influence the eosinophilic response but through various and potentially complex paths.

The first missing evidence relates to the potential effects of LT modifiers on bone marrow or in situ eosinophil progenitors and committed precursors (45). It is possible, if not likely, that these drugs exert anti-inflammatory properties at the level of the bone marrow. Studies aimed at examining the mode of action of these agents in cultures of $CD34^+$ cells obtained either from bone marrow or other hemopoietic tissue (including umbilical cord blood) in vitro (46) are needed. In addition, the effects of these agents on bone marrow levels of eosinophil-sensitive chemokines (such as RANTES and eotaxin) require investigation. This would help determine whether there is a potential blocking action of these agents on the proximal arm controlling the egress of eosinophils from hemopoietic sites (Figure 1).

Second, there is a strong likelihood that the pharmacological effects of montelukast on eosinophil numbers may be an indirect one. I prefer this hypothesis in favour of the notion that cysteinyl LTs exhibit eosinophilotactic properties. Previous and carefully conducted studies concluded that LTC_4, D_4 and E_4 had negligible chemotactic activity for eosinophils (13,47). Furthermore, these sulphidopeptide LTs showed no upregulatory effects on eosinophil effector function (including cytotoxicity), at least compared with LTB_4 (48). Thus, it seems more likely that this indirect effect on eosinophil recruitment to the airway, as shown by reduced eosinophil counts in sputum, may be mediated via LT receptor-mediated effects on epithelial cells. This may in turn influence the synthesis, storage and release of eosinophilotactic chemokines, such as RANTES and eotaxin (49,50), both of which have been shown to be present in the bronchial tissue in asthma (51,52). In addition to chemokines, epithelial cell-induced eosinophil chemoattraction may also be influenced by cytokines, particularly IL-16 (Figure 1). IL-16, a potent T-cell and eosinophilotactic cytokine and a major product of bronchial epithelial cells, as well as other inflammatory cells including eosinophils, uses CD4 receptors on eosinophils in its chemotactic activity (53,54). IL-16 expression has recently been shown to be a pathological feature of human bronchial asthma (55).

Third, it seems that we know very little about the influence of montelukast treatment on IL-5 bioactivity both locally and systemically. This cytokine is a critical factor in eosinophil terminal differentiation and, together with IL-3 and GM-CSF, prolongs eosinophil survival in the tissue. However, data from a recent study (42) have suggested that IL-5 levels may be reduced in the sputum. This may provide further support to the notion that LT modifiers may exert their influence on the eosinophilic response via IL-5 protein synthesis and turnover in asthmatic airways (Figure 1).

Finally, montelukast-induced reduction in the number of peripheral blood and airway eosinophils may be due to the ability of this agent to induce eosinophil death, particularly apoptosis. Furthermore, whether engaging putative Cys-LT1 on eosinophils may be important in prolonging the survival of these cells is another worthwhile area of investigation.

CONCLUSIONS

The eosinophil may be an important target for the pharmacological activities of LT receptor antagonists. It is likely that the anti-asthma efficacy of these antagonists may relate partly to their direct and/or indirect anti-eosinophilic properties. However, much remains to be unveiled in regard to their anti-eosinophilic effects. Our aim should be to obtain a better understanding of the mode of action of leukotriene modifiers with a view to clarify their potential ‘anti-inflammatory’ and "steroid-sparing" properties. Such data should contribute to a more efficacious administration of these therapies in asthma.

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