

Leukotriene E₄: Perspective on the forgotten mediator

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Leukotriene (LT) E₄ mediates many of the principal features of bronchial asthma, such as bronchial constriction, hyperresponsiveness, eosinophilia, and increased vascular permeability. Furthermore, it is the most stable of the cysteinyl leukotrienes (CysLTs) and can be active at the site of release for a prolonged time after its synthesis. There might be several reasons why LTE₄ has been forgotten. LTE₄ demonstrated low affinity for CysLT₁ and CysLT₂ receptors in equilibrium competition assays. It was less potent than other CysLTs in functional assays, such as calcium flux, in cells transfected with CysLT₁ and CysLT₂. The introduction of CysLT₁ antagonists into clinical practice diverted interest into CysLT₁-related mechanisms, which were mediated mainly by LTD₄. However, experiments with animal models and human studies have revealed that LTE₄ has unique characteristics that cannot be explained by the current knowledge of CysLT₁ and CysLT₂. These activities include its potency relative to other CysLTs to increase airway responsiveness to histamine, to enhance eosinophilic recruitment, and to increase vascular permeability. Asthmatic airways also demonstrate marked *in vivo* relative hyperresponsiveness to LTE₄, especially in patients with aspirin-sensitive respiratory disease. This has stimulated a search for additional LT receptors that would respond preferentially to LTE₄ stimulation. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Cysteinyl leukotriene, leukotriene E₄, asthma, aspirin-sensitive respiratory disease, cysteinyl leukotriene receptor

The cysteinyl leukotrienes (CysLTs) were discovered during the characterization of a mixture of compounds referred to as the slow-reacting substances of anaphylaxis (SRS-As). By the 1960s and 1970s, many of the biologic and physiochemical properties of the SRS-As, in particular their smooth muscle-contracting abilities, had been described. It was the lasting contractile property of SRS-As at very low concentrations that generated interest in these lipid compounds as putative mediators in bronchial asthma. By

Abbreviations used

ASRD: Aspirin-sensitive respiratory disease
CysLT: Cysteinyl leukotriene
LT: Leukotriene
SRS-A: Slow-reacting substance of anaphylaxis
uLTE₄: Urinary LTE₄

the 1980s, the covalent structure and total synthesis of SRS-As had been determined, and SRS-As were discovered to be a mixture of 3 arachidonic acid derivatives, leukotriene (LT) C₄ and its 2 metabolites, LTD₄ and LTE₄.¹⁻³

The elucidation of the structures and synthetic pathways for the LTs was soon followed by a comprehensive assessment of their biologic functions. Most of the early studies focused on LTC₄ and LTD₄ and demonstrated that both compounds were very potent inducers of contraction in guinea pig airways and isolated human bronchi.^{4,5} *In vivo* studies in healthy individuals confirmed that LTC₄ and LTD₄ when inhaled were thousands of times more potent than histamine in inducing airway contraction.^{6,7} The biologic activities of LTE₄ have generally been studied much less, probably because it was an end product of CysLT metabolism and demonstrated lower bronchoconstrictor potency in comparison with LTC₄ and LTD₄.⁸

This review summarizes some of the activities of LTE₄ and re-examines some results of earlier studies, many of which are in human subjects, suggesting that LTE₄ is a potent mediator in its own right.

LTE₄ AND BRONCHIAL HYPERRESPONSIVENESS

Allergen challenge of lung tissue from asthmatic subjects elicits bronchial contraction that correlates with the release of LTC₄, LTD₄, and LTE₄.⁹ It is interesting that all CysLTs were equally potent agonists for contraction of human bronchi in this early study. LTE₄ has also been shown to be equally effective as LTC₄ and LTD₄ in contracting human intralobar airways, suggesting that LTE₄ should be considered as a full agonist for human bronchial contraction.¹⁰ These observations were confirmed *in vivo* by Davidson et al,⁸ who demonstrated that LTE₄ is a potent bronchoconstrictor in human subjects.

Thus LTE₄ is similar to LTC₄ and LTD₄ in its bronchoconstrictor activity but, in contrast, has a unique relationship with “nonspecific” hyperresponsiveness in asthma. Although in healthy subjects inhaled LTC₄ and LTD₄ were several thousand times more potent than histamine in eliciting comparable impairment of pulmonary function, in asthmatic subjects inhaled LTC₄ and LTD₄ were only hundreds of times more potent, showing that the relative responsiveness to these 2 CysLTs when inhaled was much greater in healthy than in asthmatic subjects.^{6,11,12} Furthermore, subjects who were most responsive to methacholine or histamine had the

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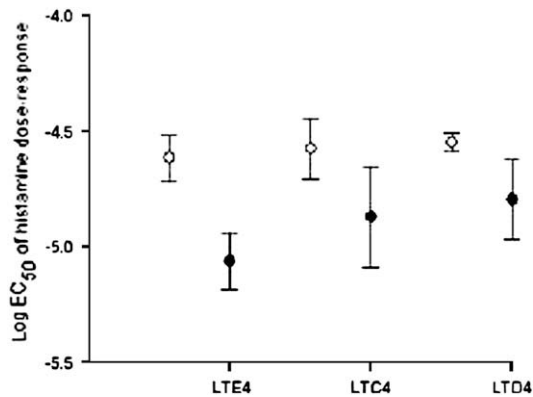


FIG 1. Log median effective concentration (EC_{50}) of histamine dose-response effect in control guinea pig tracheas (open circles) and tracheas pre-treated (solid circles) with 23 mol/L LTE_4 , 80 mol/L LTC_4 , and 40 mol/L LTD_4 . LTE_4 ($n = 8$) significantly enhanced the responsiveness to histamine of tracheal smooth muscle ($P < .01$), whereas LTC_4 ($n = 7$) and LTD_4 ($n = 7$) did not elicit increased responsiveness to histamine. Reproduced with kind permission from Lee et al.¹⁶ Copyright PNAS.

lowest relative airway response to both of these LTs.¹³ Of interest, the lower relative airway responsiveness to CysLTs in patients with asthma was not observed when LTE_4 was analyzed. Asthmatic subjects were 26 times more sensitive to inhaled LTE_4 than healthy subjects, whereas they were only 7 times more sensitive to methacholine and histamine, suggesting that asthmatic airways might be selectively hyperresponsive to LTE_4 in contrast to other CysLTs.¹⁴ In addition, there was a positive correlation between airway responsiveness to LTE_4 and responsiveness to histamine and methacholine in asthmatic subjects, emphasizing a unique property of LTE_4 in patients with asthma. This observation was confirmed and extended in a subsequent study in which the relative potencies of LTC_4 , LTD_4 , and LTE_4 were directly compared with those of histamine and methacholine in the same healthy and asthmatic subjects.¹⁵ There was a substantially augmented level of hyperresponsiveness to LTE_4 in subjects with asthma, which was not observed for the other bronchoconstrictor agents, when compared with that seen in healthy subjects. Compared with healthy subjects, the airways of a group of asthmatic subjects were on average only 14-fold more responsive to inhaled histamine, 16-fold more responsive to methacholine, 6-fold more responsive to LTC_4 , and 9-fold more responsive to LTD_4 but 219-fold more responsive to LTE_4 . It was suggested that the mechanism of the bronchoconstriction induced by LTE_4 might be distinct from that produced by LTC_4 and LTD_4 in subjects with asthma, reflecting CysLT receptor heterogeneity in human bronchi.¹⁵

LTE_4 is also unusual among many other bronchoconstricting agents in its potential to enhance airway hyperresponsiveness to other agonists. It was first demonstrated in a guinea pig model that LTE_4 , but not LTC_4 and LTD_4 , enhanced the contractile response of tracheal spirals to histamine.¹⁶ The ability of LTE_4 to induce hyperresponsiveness to histamine was unrelated to contractile activity because LTC_4 and LTD_4 did not elicit increased responsiveness to histamine despite constricting tracheal tissues by the same degree as LTE_4 (Fig 1). Indomethacin abolished the LTE_4 -induced hyperresponsiveness to histamine but did not alter CysLT-induced contraction, thereby dissociating contraction from the capacity to enhance histamine responsiveness. It also suggested that the enhancement of airway reactivity is related to the generation of COX pathway products, whereas CysLT-

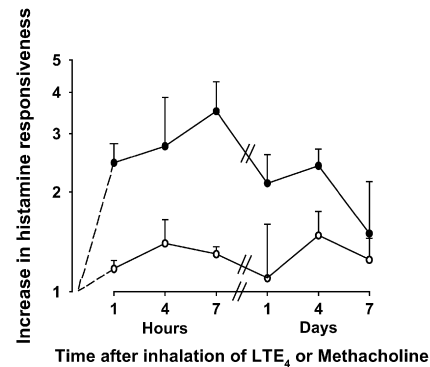


FIG 2. Time course of changes in airway histamine responsiveness after inhalation of bronchoconstricting doses of methacholine (open circles) and LTE_4 (solid circles). Results are the geometric mean $\pm$ SEM for 4 subjects with asthma and were calculated as the ratio of the provocative dose of histamine needed to cause a 35% fall in specific airway conductance (PD_{35}) after inhalation of diluent to histamine PD_{35} after inhalation of methacholine or LTE_4 . After inhalation of LTE_4 , there was a time-dependent increase in histamine responsiveness that increased to a maximum of 3.5-fold at 7 hours after challenge and disappeared by 1 week. Reproduced with kind permission from Arm et al.¹⁸ Copyright Elsevier.

elicited contraction is mediated by a mechanism independent of prostaglandin synthesis. CysLT-induced contraction was inhibited by 1 μ g/mL of the SRS-A inhibitor FPL55712, whereas a 10 times higher concentration of FPL55712 was required to inhibit LTE_4 -induced augmentation of responsiveness to histamine, suggesting that LTE_4 -induced hyperresponsiveness and contraction might be mediated by different receptors.¹⁶

Studies on guinea pig tissues were confirmed in isolated human bronchus. Pretreatment of human airways with LTE_4 *in vitro* enhanced histamine responsiveness, as demonstrated by a 4-fold leftward displacement of the histamine dose-response curve.¹⁷ This effect was blocked by the thromboxane A_2 receptor antagonist GR32191, suggesting that LTE_4 -induced hyperresponsiveness to histamine in human airways is also mediated by the secondary generation of COX products. These *in vitro* observations were confirmed *in vivo* by Arm et al,¹⁸ who reported that inhalation of LTE_4 in asthmatic but not healthy subjects elicited a time-dependent enhancement in histamine bronchial responsiveness that reached a maximum level at 7 hours after LTE_4 inhalation and could persist for up to 1 week (Fig 2). The capacity of LTE_4 to enhance airway responsiveness was specific for histamine because similar changes were not observed after methacholine challenge.^{18,19} These findings suggested that LTE_4 -mediated enhancement of bronchial reactivity might be agonist specific and possibly involved regulation of histamine receptor expression rather than a change in general nonspecific bronchial responsiveness. This hypothesis was supported by the finding that LTE_4 can prime histamine responses by increasing surface histamine H_1 receptor expression.²⁰

The reason for the differences in response to LTE_4 between healthy and asthmatic subjects could be related to the differences in baseline airway caliber and the possible effect of this on the deposition of LTE_4 in the bronchi. However, the observation is equally consistent with the existence of increased expression of a putative LTE_4 -specific receptor or signaling pathway in asthmatic subjects. A direct interaction between a putative LTE_4 receptor and histamine receptors or interaction at the level of intracellular signaling might be responsible for the experimentally observed phenomenon.

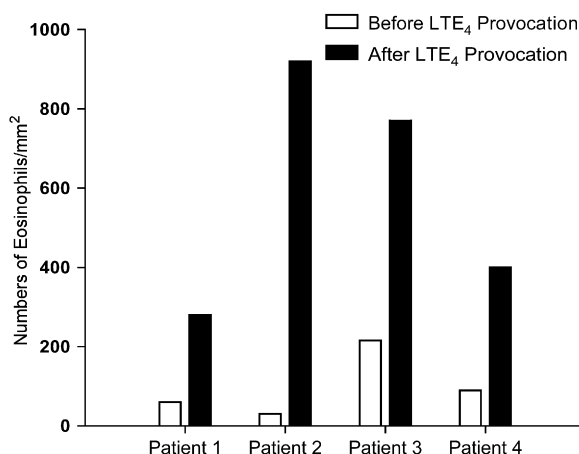


FIG 3. Changes in the number of eosinophils in the airways of 4 subjects with asthma after inhalation of LTE₄. LTE₄ elicited an increase in eosinophil numbers in the lamina propria of the airway mucosa 4 hours after inhalation in 4 asthmatic subjects. Adapted from Latinen et al.³⁰ Copyright Elsevier.

LTE₄ AND ASPIRIN-SENSITIVE RESPIRATORY DISEASE

Aspirin-sensitive respiratory disease (ASRD) is a term describing a chronic inflammatory disease of the respiratory tract characterized by asthma, chronic eosinophilic rhinosinusitis, and nasal polyposis. Patients experience acute worsening of asthma and rhinosinusitis after ingestion of aspirin or other drugs that inhibit COX-1. Even in the absence of aspirin, aspirin-sensitive patients are more likely to have moderate-to-severe asthma. They are more often prescribed rescue oral steroids and are admitted more frequently to intensive care units. They often require repeated nasal polypectomies. The airways of patients with ASRD show features of persistent inflammation, with enhanced eosinophilia and epithelial disruption.²¹ The distinct clinical features of ASRD led to speculation about fundamental differences in its pathogenesis compared with other phenotypes of asthma, and the CysLTs, particularly LTE₄, were considered to have major importance in its pathogenesis.

LTE₄ is the most stable of the CysLTs, and urinary LTE₄ (uLTE₄) is used as an index of total production of CysLTs. Between 4% and 6% of systemically administered LTC₄ appears in the urine as LTE₄ in the first 4 hours after administration.²² Christie et al²³ measured uLTE₄ levels in aspirin-sensitive and aspirin-tolerant individuals before and after challenge with aspirin and placebo. Mean resting uLTE₄ levels were on average 6-fold higher in individuals affected with ASRD compared with those seen in control asthmatic subjects. Oral aspirin challenge in the aspirin-sensitive cohort induced a 21% mean decrease in FEV₁, with an associated 4-fold increase in uLTE₄ levels. No such increase in uLTE₄ levels was measured in the control subjects after aspirin ingestion. Inhalation challenge with lysine aspirin also had a similar effect.²⁴ The increase in CysLT levels in all body fluids studied after aspirin challenge indicates upregulation of the 5-lipoxygenase pathway in ASRD that is further enhanced after exposure to nonsteroidal anti-inflammatory drugs. Furthermore, patients with ASRD have a marked airway sensitivity to inhaled LTE₄.²⁵ LTE₄ was 1870 times more potent relative to histamine in inducing a bronchoconstrictor response compared with only 145 times more potent in aspirin-tolerant asthmatic individuals.

This specific LTE₄ hyperresponsiveness in aspirin-sensitive individuals was not observed for LTC₄.²⁶

Individuals with ASRD who do not respond to conventional medical treatment can be desensitized with oral aspirin, which improves clinical outcomes and systemic corticosteroid use in many aspirin-sensitive individuals.²⁷ Desensitization also induces a highly significant reduction in airway LTE₄ hyperresponsiveness. Arm et al²⁵ demonstrated a mean 33-fold reduction in the responsiveness of the airways to LTE₄ relative to histamine within 24 hours of aspirin desensitization. The reduction in LTE₄ responsiveness persisted for as long as the patients remained desensitized by taking 600 mg of oral aspirin daily but vanished as soon as oral aspirin was stopped and aspirin sensitivity returned. Successful aspirin desensitization occurred despite substantial ongoing production of LTE₄ on aspirin challenge, suggesting that blunting of the airway responsiveness to LTE₄ might explain the efficacy of aspirin desensitization.²⁸ Effective aspirin desensitization is also associated with a decrease in the number of inflammatory cells expressing the CysLT₁, but not the CysLT₂, receptor.²⁹

LTE₄, LEUKOCYTE RECRUITMENT, AND THE SKIN

Latinen et al³⁰ demonstrated that LTE₄ inhalation led to a selective recruitment of eosinophils and neutrophils to the airway mucosa of asthmatic subjects (Fig 3). The numbers of recruited eosinophils were on average 10-fold greater than those of neutrophils, suggesting that LTE₄ is a potent eosinophil chemoattractant. These observations were supported by another study in which inhaled LTE₄ doubled the number of eosinophils in the airway mucosa in patients with mild asthma.³¹ Inhaled LTD₄ increased sputum eosinophils in asthmatic subjects in one study³² but not in another.³³ When the effects of inhaled LTD₄ and LTE₄ on eosinophil infiltration were directly compared, only LTE₄ caused a significant increase in the number of eosinophils in the lamina propria of patients with asthma.¹⁹ Thus among CysLTs, LTE₄ appears to have the most activity for attracting eosinophils to inflammatory sites in asthmatic individuals.

When equimolar concentrations of LTC₄, LTD₄, and LTE₄ were injected into the skin of healthy volunteers, all CysLTs elicited comparable wheal-and-flare reactions that peaked at 1 to 2 hours and were resolved by 4 to 6 hours, showing that LTE₄ and other CysLTs could induce cutaneous vasodilatation with edema formation.³⁴ Similar reactions were observed in patients with chronic idiopathic urticaria in whom each of the CysLTs produced a concentration-dependent, slowly developing wheal-and-flare response that was maximal between 1 and 3 hours and was still present at 4 hours. The skin response was not significantly different between each of the CysLTs or between healthy subjects and subjects who have chronic idiopathic urticaria. LTE₄ is therefore as potent as LTC₄ and LTD₄ in increasing vascular permeability and vasodilatation.³⁵

The capacity of LTE₄ uniquely to recruit eosinophils and its potency to elicit vasoactive cutaneous responses relative to other CysLTs cannot be explained by the pharmacology of the established receptors for CysLTs and strongly points to the existence of a novel LTE₄-specific receptor or receptors.

LTE₄ AND CysLT RECEPTORS

The above observations identify LTE₄ as an important mediator of the principal features of bronchial asthma, such as bronchial

TABLE I. Binding affinities of CysLTs for CysLT₁ and CysLT₂ measured in competitive radioligand binding assays^{37,38}

	IC ₅₀ (nmol/L), mean	
	CysLT ₁	CysLT ₂
LTC ₄	353	3.35
LTD ₄	0.9	3.48
LTE ₄	190.5	693

IC₅₀, Concentration of competing ligand that displaces 50% of the specific binding of the radioligand.

TABLE II. Functional potencies of CysLTs measured by using melanophore pigment dispersion and aequorin assays for CysLT₁ and CysLT₂, respectively^{37,38}

	EC ₅₀ (nmol/L), mean	
	CysLT ₁	CysLT ₂
LTC ₄	21	67
LTD ₄	0.4	104
LTE ₄	212	2300

EC₅₀, Concentration eliciting 50% of its maximal effect.

Constriction, hyperresponsiveness, eosinophilia, and increased vascular permeability. Furthermore, at least half of the LTC₄ instilled into the asthmatic airway is converted to LTE₄ within 15 minutes.³⁶ Thus in comparison with LTC₄ and LTD₄, LTE₄ is the most stable of the CysLTs and might be active for a prolonged time after its synthesis.

There might be several reasons why LTE₄ has been forgotten. After the cloning of the human CysLT₁³⁷ and CysLT₂³⁸ receptors, the relative affinities of the CysLTs to bind to their receptors were determined (Tables I and II). LTD₄ had the highest binding affinity for CysLT₁, whereas LTC₄ and LTD₄ bound the CysLT₂ receptor with equal affinity. LTE₄ demonstrated low affinity for both receptors in equilibrium competition assays. LTD₄ effectively activated calcium flux in cells transfected with CysLT₁ in a concentration-dependent manner, LTC₄ was also a full agonist but was 10 times less potent than LTD₄, and LTE₄ behaved as a weak agonist. In CysLT₂-transfected cells, LTC₄ and LTD₄ were equipotent agonists, whereas LTE₄ presented only partial agonist activity. A very similar rank order of potency was observed for CysLT-induced calcium flux in human primary cells, in elutriated monocytes, and in human umbilical endothelial cells, which dominantly expressed CysLT₁ and CysLT₂, respectively.^{39,40} The weak agonist activity of LTE₄ for CysLT₁ and CysLT₂ probably resulted in it being sidelined in studies of CysLT biology. Furthermore, the successful introduction of CysLT₁ antagonists into clinical practice with their ability to improve lung function, decrease the need for β -adrenergic agonists, attenuate cellular inflammation, and result in fewer symptoms of asthma also diverted interest into CysLT₁-related mechanisms, which were mediated mainly by LTD₄.⁴¹

The demonstration of some unique characteristics of LTE₄ and its marked relative airway hyperresponsiveness *in vivo* in human subjects that could not be explained by current knowledge of CysLT₁ and CysLT₂ stimulated a search for additional LT receptors, in particular receptors that would respond preferentially to LTE₄ stimulation. Alternative splicing of gene transcripts is one of the physiologic mechanisms that allows for expression of alternative forms of receptors with potentially unique activities. In an attempt to identify new forms of CysLT receptors, gene structures were characterized for human CysLT₁⁴² and CysLT₂.⁴⁰ Several

alternatively spliced transcripts for the genes encoding CysLT₁ and CysLT₂ were found in human primary cells. The genes were variably spliced in the 5'-untranslated region, a feature shared with many other G protein-coupled receptors, but all the identified transcripts encoded the same proteins that had previously been described. Alternative splicing of CysLT₁ and CysLT₂ cannot therefore explain the observed bias in LTE₄ responsiveness.

The recent compelling evidence for the existence of a separate LTE₄ receptor or receptors might finally provide a cogent mechanistic explanation for many of the physiologic and pharmacologic observations described in this review.⁴³⁻⁴⁵ This exciting discovery not only provides an opportunity to advance substantially the understanding of CysLT biology and CysLT receptors but offers the real possibility of developing novel therapeutics.

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