

Molecular mechanisms in allergy and clinical immunology

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The cellular biology of eosinophil eicosanoid formation and function

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Eosinophils are capable of generating eicosanoid derivatives of arachidonic acid by means of cyclooxygenase and the 5- and 15-lipoxygenase (LO) pathways. Moreover, eosinophils, because of their expression of leukotriene (LT) C₄ synthase, are a major source of 5-LO-derived cysteinyl LTs, which are potent paracrine mediators of bronchial obstruction and inflammation pertinent to asthma. The regulation of eicosanoid formation within eosinophils involves activation of key enzymes at specific intracellular sites. Calcium ionophore-elicited translocation of 5-LO to the membranes of the nuclear envelope is associated with LTC₄ formation. In addition, lipid bodies, the formation of which is initiated by specific receptor-mediated signaling pathways, are sites of cyclooxygenase- and LO-pathway eicosanoid formation. Newly formed LTC₄ can be immunolocalized at perinuclear membranes in ionophore-activated eosinophils and at lipid bodies in CCR3 agonist (eg, eotaxin) chemokine-stimulated eosinophils. The local generation of eicosanoids at distinct sites within eosinophils may be important for the roles of these eicosanoids, both as paracrine mediators pertinent to inflammation and as intracrine signal-transducing mediators that help regulate cellular responses of eosinophils. (*J Allergy Clin Immunol* 2002;109:393-400.)

Key words: Eosinophils, leukotrienes, prostaglandins, arachidonic acid, lipid bodies, perinuclear membrane

Lipid mediators, including leukotrienes (LTs), PGs, and platelet-activating factor (PAF), are major participants in the pathogenesis of asthma and other forms of allergic inflammation.¹ Eosinophils, one of the principal cell types recruited to and activated at sites of allergic inflammation, are capable of elaborating lipid mediators, including several eicosanoids derived from the oxidative metabolism of arachidonic acid (AA).² The enzymatic pathways by which eosinophils synthesize their potential

Abbreviations used

AA:	Arachidonic acid
COX:	Cyclooxygenase
cPLA ₂ :	Cytosolic phospholipase A ₂
cysLT:	Cysteinyl leukotriene
FLAP:	5-Lipoxygenase activating protein
HETE:	Hydroxyeicosatetraenoic acid
HPETE:	Hydroperoxyeicosatetraenoic acid
LO:	Lipoxygenase
LT:	Leukotriene
PAF:	Platelet-activating factor
PL:	Phospholipase

eicosanoids have been established, but the mechanisms that physiologically regulate eosinophil eicosanoid formation remain to be fully defined. Understanding the mechanisms governing eosinophil eicosanoid synthesis is important for a number of reasons. First, potentially activated eosinophils may elaborate greater quantities of eicosanoids, such as LTC₄, than normal eosinophils.^{2,3} These activated eosinophils thus are primed for enhanced LTC₄ generation in response to subsequent stimuli. Some recognized priming stimuli are chemoattractants (eg, eotaxin⁴ and PAF⁵) that may participate in the recruitment of eosinophils to sites of allergic inflammation. The mechanisms by which chemoattractants and other activating cytokines (eg, IL-5⁶) or extracellular matrix components (eg, fibronectin⁷) enhance eosinophil eicosanoid formation are pertinent to the functions of these eicosanoids as paracrine mediators of allergic inflammation. Second, some eosinophil-derived eicosanoids may be active in downregulating inflammation^{8,9}; however, the mechanisms for the differential formation and release of proinflammatory and anti-inflammatory eicosanoids have not been delineated. Third, it is increasingly likely that eicosanoids synthesized within cells, including eosinophils, may have intracellular (eg, intracrine) roles in regulating cell functions in addition to the more recognized activities of eicosanoids as paracrine mediators of inflammation.

As in other cells, activation of pathways for enhanced eicosanoid formation can be facily elicited experimentally in eosinophils with Ca⁺⁺ ionophores (eg, A23187),

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which increase intracellular calcium. This activation mechanism was important in delineating the enzymatic pathways of eicosanoid synthesis active in eosinophils, as in other cells. Ca^{++} ionophore activation, however, is supraphysiologic and may not be indicative of eicosanoid synthesis elicited by more physiologic stimuli. In eosinophils the principal product of the 5-lipoxygenase (5-LO) pathway of AA metabolism is LTC_4 , the formation of which is tightly coupled to the 5-LO catalyzed formation of LTA_4 (as evidenced by the paucity of nonenzymatic breakdown products of LTA_4 formed in eosinophils in contrast to neutrophils).¹⁰ In neutrophils the principal 5-LO product is LTB_4 , the formation of which from LTA_4 may occur within the cytoplasm or even extracellularly.^{11,12} Thus in eosinophils the recently described methodology to immunolocalize intracellular sites of LTC_4 formation can provide direct evidence of the intracellular sites of 5-LO activation.¹³ Alternatively, 5-LO protein may be immunolocalized at specific intracellular sites, although such localization might not necessarily assess the enzymatic activity of the localized 5-LO enzyme. In this review we consider the evolving understanding of the potential roles of intracellular domains in regulating eicosanoid formation and function within eosinophils.

PATHWAYS OF EOSINOPHIL EICOSANOID FORMATION

In human eosinophils the oxidative metabolism of AA may occur through pathways mediated by 2 cyclooxygenases (COXs; PGH synthase) and 2 LO enzymes. COX catalyzes 2 reactions, a COX reaction that inserts 2 molecules of oxygen into substrate AA to form PGG_2 and a subsequent endoperoxidase reaction that reduces PGG_2 to its 15-hydroxy analogue, PGH_2 . COX-pathway eicosanoids produced by human eosinophils include thromboxane B_2 and PGE_2 , with lesser quantities of PGD_2 and 6-keto- $\text{PGF}_{1\alpha}$.¹⁴⁻¹⁶ Both COX enzymes, the nominally constitutive COX-1 and the potentially inducible COX-2, can be present in eosinophils. What regulates specific COX-2 induction in eosinophils is not yet defined, although eosinophils (and other leukocytes) in the airways of asthmatic subjects exhibit immunostaining for COX-2 protein.^{17,18}

Eosinophils contain 2 LO enzymes, 5-LO and 15-LO. 5-LO catalyzes 2 reactions. 5-LO inserts one oxygen molecule into the 5 position of AA to form 5S-hydroperoxy-eicosatetraenoic acid (HPETE) and transforms 5-HPETE into an unstable allylic epoxide, LTA_4 . In species other than guinea pigs,² the subsequent metabolism of LTA_4 differs between eosinophils and neutrophils. In neutrophils LTA_4 hydrolase enzymatically hydrolyses LTA_4 to LTB_4 . In contrast, in eosinophils a specific glutathione S-transferase, LTC_4 synthase, catalyzes the adduction of glutathione to LTA_4 to form LTC_4 .¹⁹ In contrast to neutrophils, eosinophils also contain 15-LO.²⁰ This enzyme catalyzes both the hydroperoxidation of AA, with the insertion of one molecule of oxygen at

position 15 to form 15-HPETE and the insertion of molecular oxygen into other polyunsaturated fatty acids and phospholipids.²¹ Eicosanoids formed by the 15-LO pathway of AA metabolism include the monohydroxy acid 15-hydroxyeicosatetraenoic acid (HETE) and the dihydroxy acids 8,15-diHETE and 14,15-diHETE, as well as a trihydroxy acid, lipoxin A_4 , requiring the additional activities of other LO enzymes.^{22,23}

Because substrate AA is not free within cells but is esterified in the sn-2 position of phospholipids, an event requisite for the oxidative metabolism of AA by any of these potential pathways in eosinophils involves the activation of phospholipase (PL) A_2 enzymes that act on arachidonyl-phospholipids resident in cell membranes.

CYSTEINYL LT SYNTHESIS AND RELEASE BY EOSINOPHILS

The cysteinyl LTs (cysLTs), LTC_4 and its extracellular derivatives, LTD_4 and LTE_4 , are recognized as paracrine mediators pertinent to asthma and allergic diseases on the basis of their receptor-mediated capabilities to elicit bronchoconstriction, mucous hypersecretion, bronchial hyperresponsiveness, increased microvascular permeability, and additional eosinophil infiltration.²⁴⁻²⁶ Eosinophils are a major source of cysLTs^{10,27} and have been identified as the principal LTC_4 synthase expressing cells in bronchial mucosa biopsy specimens of asthmatic subjects.²⁸ Hence much interest in understanding the regulation of eicosanoid formation in eosinophils has focused on the mechanisms that regulate eosinophil cysLT formation and release.

The regulated synthesis of cysLTs is initiated by PLA_2 -mediated release of AA from membrane phospholipids. In human eosinophils activated with N-formyl-methionyl-leucyl-phenylalanine, an 85-kd cytosolic PLA_2 (c PLA_2), and not secretory PLA_2 or Ca^{++} -independent PLA_2 , is responsible for releasing AA for LTC_4 formation.²⁹ As in other cells, released AA binds to 5-LO-activating protein (FLAP), enabling it to be acted on by 5-LO to form LTA_4 . LTC_4 synthase then catalyzes the formation of LTC_4 . LTC_4 is actively transported across the plasma membrane by a probenecid-sensitive carrier-mediated mechanism to function as a paracrine mediator.³⁰ Extracellularly, LTC_4 is processed by means of sequential enzymatic cleavage of its glutamic acid and glycine residues to form LTD_4 and LTE_4 , respectively.

The synthesis of cysLTs in eosinophils can be governed at several steps, some of which may involve enhanced synthesis of relevant proteins (eg, 5-LO and FLAP) or phosphorylation of key enzymes. Whether 5-LO becomes phosphorylated in eosinophils, as in other cells, is not known.³¹ Eosinophil c PLA_2 can be regulated both by means of phosphorylation and increases in intracellular Ca^{++} .^{29,32} In addition to these biochemical regulators of cysLT formation, as emphasized by Peters-Golden and Brock,^{33,34} the regulated

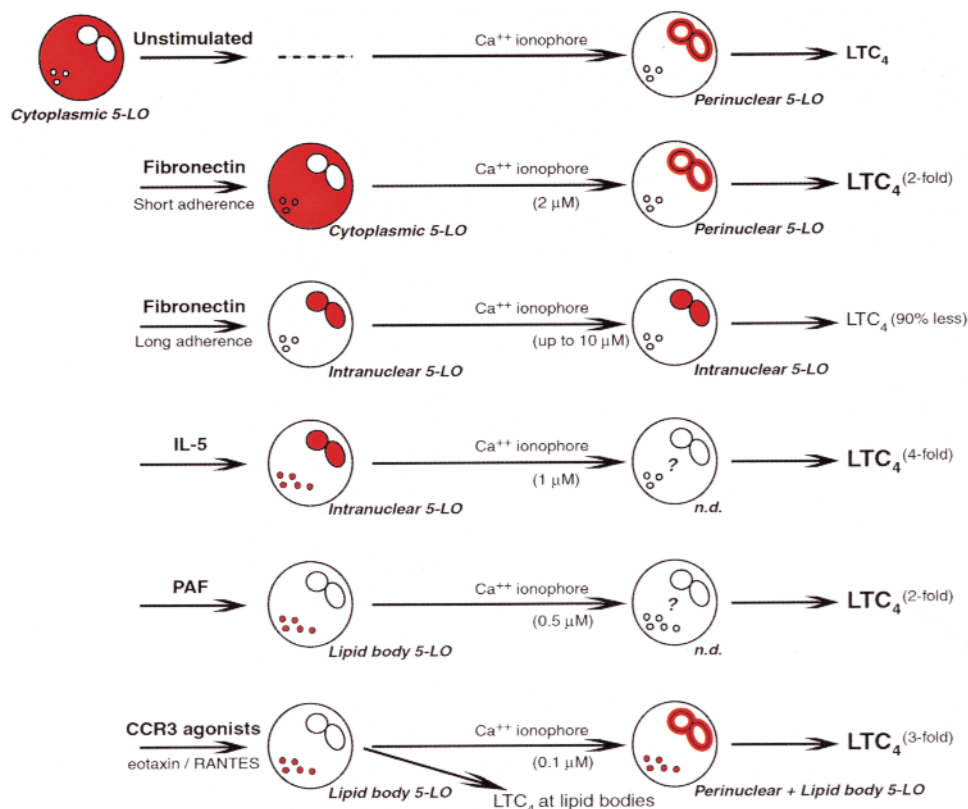


FIG 1. Schematic summary of studies evaluating 5-LO localization within human eosinophils. Intracellular sites of immunolocalized 5-LO protein within eosinophils, highlighted in red, changed from primarily cytosolic to different compartments in response to varied priming agents and subsequent activation by varied concentrations (range, 0.1–10 $\mu mol/L$) of calcium ionophore (A23187). LTC_4 released from eosinophils was detected by means of ELISA. *n.d.*, Not determined.

localization and expression of LT synthesis at specific intracellular compartments are important in governing LT formation and, likely, LT functions. Thus the activation of eicosanoid-forming enzymes at specific membranous intracellular sites within eosinophils, including perinuclear membranes and cytoplasmic lipid bodies, is critical to the formation of cysLTs and other eicosanoids by eosinophils.

EOSINOPHIL PERINUCLEAR MEMBRANES

Whereas plasma membranes were initially assumed to be the sites of paracrine-mediator eicosanoid synthesis in leukocytes, the findings that the integral membrane protein FLAP and 5-LO were localized by means of immunogold electron microscopy to perinuclear membranes in activated human neutrophils and monocytes directed attention to the nuclear envelope as sites of LT synthesis.³⁵ Subsequently, LTC_4 synthase, an integral membrane protein homologous to FLAP, was also localized to the nuclear envelope.³⁶ In contrast, 5-LO in resting neutrophils and monocytes is resident in the cyto-

plasm but is found within the nucleus of alveolar macrophages and mast cells.³³ A23187-induced rises in intracellular Ca^{++} translocate 5-LO to the nuclear envelope in these cells.³³

In human eosinophils isolated from both normal and mildly atopic donors, 5-LO was found predominantly within the cytosol (and also at lipid bodies).^{7,37,38} Brock et al⁷ evaluated the perinuclear translocation of eosinophil 5-LO and its correlation with LTC_4 release (Fig 1).⁷ After activation of eosinophils with A23187 (2 $\mu mol/L$), 5-LO protein was immunolocalized in a perinuclear pattern, likely in the nuclear envelope and endoplasmic reticulum.⁷ This perinuclear localization of 5-LO protein was fully in accord with the more recent immunolocalization of newly formed 5-LO-derived LTC_4 to the perinuclear membranes in eosinophils stimulated only with A23187 (0.1 $\mu mol/L$).¹³ In eosinophils that adhered briefly (5 minutes) to fibronectin, stimulation with N-formyl-methionyl-leucyl-phenylalanine and cytochalasin B yielded both perinuclear and lipid body localization of 5-LO, and stimulation with 2 $\mu mol/L$ A23187 yielded about 2-fold more LTC_4 release than found with nonadherent

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FIG 2. Immunogold localization of 5-LO within lipid bodies of unstimulated eosinophils from a donor with hypereosinophilic syndrome shows gold labeling, indicating 5-LO in lipid bodies (**A** and **C**), which was absent from control 5-LO-absorbed anti-5-LO antisera (**B**) or nonimmune sera (**D**). Lipid bodies are surrounded by a monolayer of phospholipids and contain a nonhomogeneously dense organization with numerous small compartments and an internal honeycomb structure. In **C**, several gold particles reside in the perinuclear cistern (*arrow*). In **A**, cytoplasmic tubules and vesicles of smooth endoplasmic reticulum adjacent to the lipid body also have gold particles associated with them (*arrows*). Images reproduced with permission from *J Exp Med*. © Copyright by The Rockefeller University Press.⁴⁴

eosinophils.⁷ Eosinophils that adhered to fibronectin for up to 2 hours demonstrated intranuclear (nucleoplasmic) redistribution of 5-LO, yet unlike 5-LO recruited into the nuclei of neutrophils, A23187 activation did not translocate the intranuclear 5-LO to the nuclear envelope of eosinophils, and correspondingly, these eosinophils released 90% less LTC₄.⁷ The means by which 5-LO remained durably associated with the nucleoplasm in fibronectin-adherent eosinophils has not yet been defined.

Cowburn et al³⁸ evaluated the effects of the eosinophilopoietic cytokine IL-5, which is known to enhance eosinophil LTC₄ release,⁶ on 5-LO localization and LTC₄ release. IL-5 not only elicited enhanced synthesis of FLAP in eosinophils but also elicited increased LTC₄ release in response to 1 μ mol/L A23187. IL-5 enhanced the nuclear localization of 5-LO (and, although not commented on by these authors, probably also in

punctate cytoplasmic lipid bodies; see their Fig 2, *D*). Potential redistribution of nucleoplasmic 5-LO to the nuclear envelope of eosinophils after A2387 activation was not assessed. 5-LO nuclear import elicited by IL-5 was suggested to contribute to IL-5's ability to enhance eosinophil LTC₄ release.³⁸

EOSINOPHIL CYTOPLASMIC LIPID BODIES

Lipid bodies are cytoplasmic organelles that may be present in all nucleated human cells. In leukocytes and other cells associated with inflammation, lipid body numbers are characteristically increased.³⁹ Lipid bodies in diverse organisms are characteristically rich in triglycerides and surrounded by a monolayer of phospholipids.^{40,41} Lipid bodies are solubilized and not recognizable in cells subjected to drying or to fixation and staining with alcohol-based reagents. In eosinophils triglyceride lipids overlie an as yet ill-understood, internal honeycomb membranous structure,³⁷ perhaps related to or derived from the endoplasmic reticulum (Fig 2). It is likely that membrane-bound proteins pertinent to eicosanoid formation (eg, LTC₄ synthase and COX) are localized within this internal membranous architecture because these proteins exhibit internal and not circumferential immunolocalization at lipid bodies.

Lipid bodies are prominent in circulating eosinophils freshly isolated from normal donors. Normal blood neutrophils contain approximately 3 lipid bodies per cell, whereas blood eosinophils from healthy donors contain approximately 9 lipid bodies per eosinophil. In vivo lipid bodies characteristically increase in size and number in eosinophils associated with various forms of eosinophilic inflammation, including those in patients with the hypereosinophilic syndrome^{42,43} and in antigen-challenged asthmatic patients (Weller PF, unpublished observations).

Several specific agonists can trigger signaling mechanisms that rapidly (within a few minutes) stimulate the formation of new lipid bodies within human eosinophils (Fig 3). PAF, acting through its G protein-linked receptor, stimulates lipid body formation and requires downstream signaling through activation of 5-LO to form 5-HETE, which itself acts through a likely G protein-linked receptor. Subsequent signaling is mediated by protein kinase C activation and is partly dependent on new gene expression and protein synthesis.^{37,44} In addition, chemokines acting through CCR3 receptors, including RANTES, eotaxin, eotaxin 2, and eotaxin 3, can initiate intracellular signaling that also culminates in de novo formation of lipid bodies.^{4,13} CCR3-initiated signaling involves downstream activation of phosphoinositide 3-kinase and the extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinases but does not depend on activation of 5-LO, protein kinase C, or tyrosine kinases or on the generation of PAF.¹³ On the other hand, activation of tyrosine kinases is involved in PAF-elicited lipid body formation in activated eosinophils from patients with hypereosinophilic syndrome and in GM-CSF-treated eosinophils.⁴³ A cell-permeable diglyceride, oleyl-acetyl-glycerol, and *cis*-unsatu-

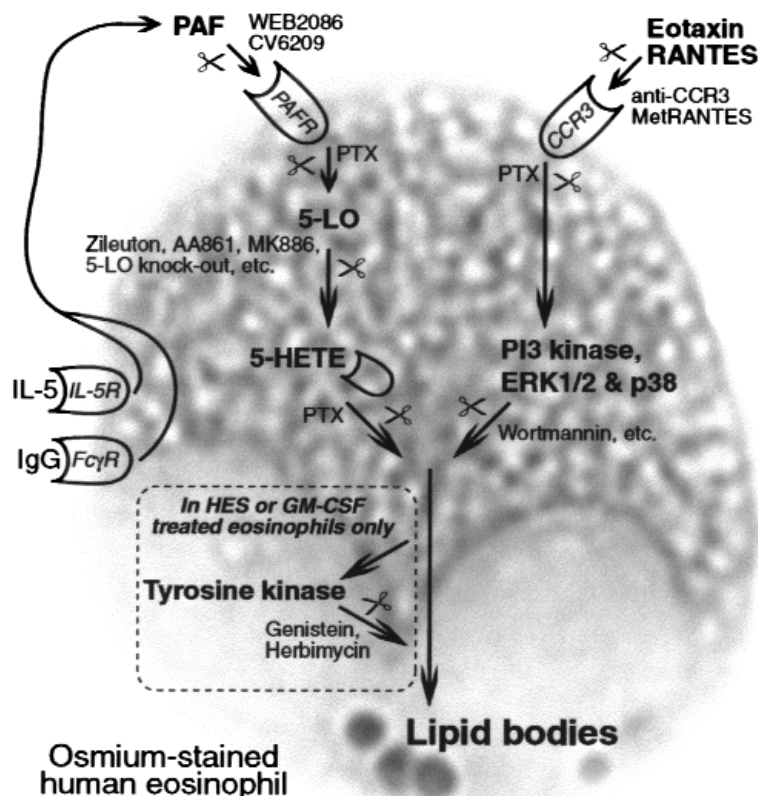


FIG 3. Cellular-signaling pathways that elicit the formation of new lipid bodies within human eosinophils. Various physiologic stimuli acting on their specific receptors, including exogenous PAF, both IL-5 and IgG immune complexes acting by means of endogenous PAF formation, and the CCR3 agonists eotaxin and RANTES are able to induce lipid body formation by activating distinct downstream kinases. *PTX*, Pertussis toxin; *ERK*, extracellular signal-regulated kinase. *Scissors symbol* denotes inhibition.

rated fatty acids, including AA and its nonesterifiable analogs, also activate pathways leading to lipid body formation in leukocytes.⁴⁵

Lipid bodies in eosinophils contain arachidonyl-phospholipids,⁴⁶ and enzymes needed to release AA from phospholipids, including cPLA₂ and activating mitogen-activated protein kinases, are also found within lipid bodies.⁴⁷ In addition, eosinophil lipid bodies contain all the enzymes needed for eicosanoid synthesis by eosinophils, including 5-LO (Fig 2), 15-LO, COX, and LTC₄ synthase.^{37,43,48}

That lipid bodies in eosinophils contribute to 5-LO- and COX-pathway eicosanoid formation is supported by findings that increases in lipid body numbers correlated with increased LTC₄ and PGE₂ release by these cells when activated with submaximal A23187. Thus eosinophils first primed to form lipid bodies with increasing concentrations of stimuli, including PAF, *cis*-unsaturated fatty acids, RANTES, and eotaxins, released correspondingly increased amounts of LTC₄ and PGE₂ on ionophore activation.^{4,13,37,45,49} Bartemes et al⁵⁰ have also demonstrated that eosinophils, in response to IL-5 or FcγR-mediated activation, generate endogenous PAF, which acts as an autocrine mediator to elicit both lipid body formation and correlative increases in LTC₄ release.

Conversely, agents that inhibit lipid body formation in vitro inhibited the priming response for enhanced LTC₄ release from eosinophils.^{37,45}

A potential role for perinuclear eicosanoid formation in these priming responses in eosinophils was unlikely based on studies of enucleated eosinophil cytoplasts.³⁷ Eosinophil cytoplasts formed lipid bodies in response to PAF stimulation, and these lipid bodies contained immunolocalized 5-LO, COX, and LTC₄ synthase. Moreover, in PAF-primed, nuclei-free eosinophil cytoplasts, the increased numbers of lipid bodies strongly correlated with the increased production of both LTC₄ and PGE₂ after stimulation with calcium ionophore.³⁷

With a new strategy to cross-link newly formed LTC₄ at its sites of synthesis within eosinophils, it has been possible with a fluorochrome-conjugated anti-cysLT antibody to immunolocalize newly formed LTC₄ within eosinophils.¹³ In eosinophils solely activated with calcium ionophore, immunofluorescent localization of newly formed LTC₄ exhibited a predominantly perinuclear localization,¹³ which is in accord with the perinuclear immunolocalization of 5-LO protein in ionophore-activated eosinophils.⁷ With eotaxin or RANTES chemokine stimulation, eosinophils not only became polarized and formed new lipid bodies but also demonstrated synthesis

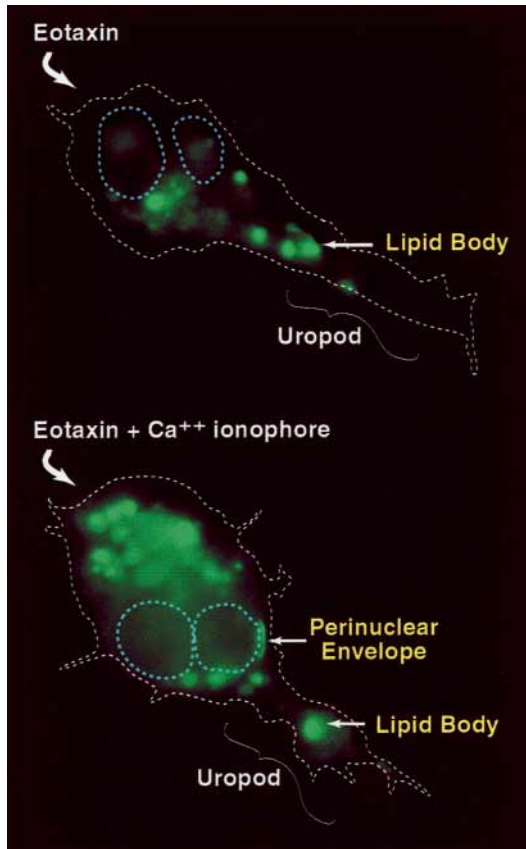


FIG 4. Cytoplasmic lipid bodies and perinuclear membranes as sites of LTC₄ synthesis in eosinophils. Intracellular immunofluorescent localization of newly formed LTC₄ (green staining) is present at cytoplasmic lipid bodies in eotaxin-primed eosinophils (*top*) and at both lipid bodies and perinuclear membranes in eotaxin-primed and A23187-challenged eosinophils (*bottom*). Eosinophil plasma membranes and perinuclear membranes have been outlined with dotted white and blue lines, respectively, to facilitate intracellular localization. Newly formed LTC₄ was cross-linked to adjacent proteins with a water-soluble carbodiimide and detected with a green fluorochrome-conjugated anti-cysLT mAb, as detailed elsewhere.¹³

of LTC₄ at lipid bodies (Fig 4, *top*), although with these chemokines alone, levels of extracellularly released LTC₄ could not be detected by means of immunoassay. When eosinophils first stimulated with these chemokines were subsequently activated with a submaximal concentration (0.1 μ mol/L) of A23187, LTC₄ formation occurred at lipid bodies and at the perinuclear membrane (Fig 4, *bottom*), and LTC₄ was released extracellularly.¹³ The phosphoinositide 3-kinase K inhibitor wortmannin blocked both the formation of extracellularly released LTC₄ and lipid body LTC₄ synthesis but not perinuclear LTC₄ synthesis.¹³ These findings provided support for the roles of lipid bodies as sites of LTC₄ synthesis both for the heightened primed release of LTC₄ in response to submaximal ionophore activation and as sites of lower level LTC₄ synthesis in eosinophils stimulated solely with eotaxin or RANTES.¹³

FUNCTIONAL CONSEQUENCES OF COMPARTMENTALIZED EICOSANOID FORMATION IN EOSINOPHILS

The capacity of eicosanoids, such as LTC₄, to be synthesized at 2 discrete sites, perinuclear membranes and lipid bodies, in eosinophils raises questions of the roles of these pools of eicosanoids in the functioning of eosinophils in inflammation. As noted above, cysLTs are well-recognized paracrine mediators pertinent to asthma and allergic inflammation, but how these eicosanoids transit from their intracellular sites of synthesis for their extracellular release, which is requisite for their paracrine functions, remains uncertain. This is especially true for LTC₄ formed at perinuclear membranes that are not proximate to the cell surface. Furthermore, it is unclear what sense it makes to use arachidonyl-phospholipids resident in perinuclear membranes as a source of substrate for forming quantities of secreted eicosanoid mediators. As evident in eotaxin-stimulated eosinophils, newly formed lipid bodies are nonrandomly distributed within activated eosinophils.¹³ Some are proximate to the nucleus, whereas others are in the uropods of polarized eosinophils, and this latter location, which is more proximate to the cell membrane, might facilitate transport-mediated secretion of newly formed LTC₄. Furthermore, lipid body arachidonyl-phospholipids might provide a source of substrate AA without requiring the perturbation of the integrity of another organelle's membranes and could be replenished from the arachidonyl-triglycerides abundant in lipid bodies.⁵¹

In addition to their potential roles as paracrine mediators, eicosanoids and related lipids are increasingly being recognized to have autocrine effects. For instance, eosinophil cysLTs exert autocrine effects to enhance eosinophil survival,⁵² and the enhanced plasma membrane expression of activation-related CD69 on human eosinophils induced by PAF- and IL-5 is dependent on endogenous eosinophil-derived 5-LO metabolites.⁵³ Endogenous 5-LO activation to form 5-HETE is required in the PAF-initiated signal transducing cascade that leads to lipid body formation (Fig 3).^{13,44} Moreover, the capacity of eotaxin to stimulate the vesicular transport-mediated release of preformed IL-4, a cytokine important to allergic responses, from eosinophil granules⁵⁴ is 5-LO dependent. In this case endogenous LTC₄, formed at eosinophil lipid bodies, acts as an intracrine signaling molecule to regulate this CCR3-elicited IL-4 release (Bandeira-Melo C and Weller PF, unpublished observations). Thus eicosanoids may act solely intracellularly as intracrine signal-transducing mediators.

Although eosinophils express the 2 known cysLT receptors,^{55,57} little is known about the plasma membrane or intracellular distribution of these or other eicosanoid receptors in eosinophils. On other cells, expression of specific PG receptors is beginning to be localized at intracellular sites, including the nuclear envelope.⁵⁸ Indeed, the localization of COX- and LO-pathway enzymes at the nuclear envelope and the nucle-

oplasm may relate less to paracrine mediator formation and more to intracrine signal-transducing activities pertinent to more local transcriptional or other nuclear functions.^{33,59,60}

CONCLUSIONS AND THERAPEUTIC IMPLICATIONS

The generation of eicosanoids within eosinophils and other cells is important not only for the role of eicosanoids as extracellular paracrine mediators of inflammation but also for their role as intracrine signal-transducing molecules that may regulate fundamental cellular responses within the nucleus or cytoplasm, such as the release of cytokines from eosinophils. Distinct intracellular domains within eosinophils (ie, perinuclear membranes and lipid bodies) are now recognized as sites of eicosanoid formation. Methodological limitations, such as supraphysiologic elicitation of eicosanoid formation with calcium ionophore and difficulties in preserving or assessing specific cellular domains, such as lipid bodies or even plasma membranes, may need to be overcome. The elicitation of specific eicosanoid formation at distinct intracellular domains within eosinophils (and other cells) will need to be more directly assessed to ascertain the mediator functions of these eicosanoids. The capability of localizing sites of LTC₄ synthesis within eosinophils at lipid bodies in both CCR3-mediated chemokine-stimulated eosinophils and CCR3-active chemokine-primed and ionophore-activated eosinophils has helped to define mechanisms for enhanced primed generation of 5-LO and COX pathway-derived paracrine mediators and for the intracrine regulatory roles of lipid body-derived LTC₄ as signal-transducing mediators, regulating eosinophil vesicle-mediated degranulation. Therapeutic agents in current use that inhibit 5-LO or cysLT1 receptors may have activities broader than simply blocking the paracrine-mediator activities of cysLTs. Furthermore, with greater understanding of the intracellular sites of eosinophil eicosanoid synthesis and eicosanoid receptor expression and of the functional consequences of such eicosanoid receptor-mediated cellular regulation, newer therapeutic agents may be targeted to regulate specific activities of eicosanoids pertinent to asthma and other allergic diseases.

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